BIOPESTICIDE REGISTRATION ACTION DOCUMENT

Bacillus thuringiensis Cry1F Corn

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

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I. Overview

Cry1F is a naturally occurring insecticidal crystal protein found in the soil bacteria, *Bacillus thuringiensis* subspecies *aizawai.*

OPP Chemical Code: 006481

Pesticide Name: *Bacillus thuringiensis* subspecies Cry1F Protein and the Genetic Material Necessary for Its Production (Plasmid Insert PHI 8999) in Corn

Trade and Other Names: Herculex™ I Insect Protection, Pioneer Brand Seed Corn with Herculex™ I

Applicants: Mycogen Seeds
c/o Dow Agrosciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054

Pioneer Hi-Bred International, Inc.
7250 NW 62nd Avenue
P.O. Box 552
Johnston, Iowa 50131-0552

Uses: Full Commercial Use in Field Corn

Regulatory History:

Current Approval
Mycogen Seeds (a Dow Agrosciences company) and Pioneer Hi-Bred International, Inc. (a Dupont company) applied to register a genetically engineered *Bacillus thuringiensis* (B.t.) corn plant-incorporated protectant that contains the *cry*1F gene and expresses the Cry1F protein. These registrations were granted on May 18, 2001 and assigned EPA Registration Numbers 68467-2 and 29964-3. The Cry1F protein protects the corn from the European corn borer, Southwestern corn borer, fall armyworm and black cutworm. The pesticide active ingredient is known as *Bacillus thuringiensis* subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in corn. Mycogen Seeds (Dow) also submitted a petition requesting an exemption from the requirement of a tolerance for all Cry1F plant-incorporated protectants, including the corn produced by the use of plasmid insert PHI 8999.

The initial registration application and tolerance petition were submitted in September of 1999. A public docket was established and notices of receipt and filing were published in the Federal Register to give the public the opportunity to comment on the pending applications. Comment periods closed on April 3, 2000 and July 24, 2000. No comments were received.

Experimental Use Permit

A crop destruct experimental use permit (68467-EUP-2) was initially granted to Mycogen Seeds in April of 1999. In response to a Federal Register notice announcing receipt of the application for this EUP, comments were submitted to the docket by the PUNA Outdoor Circle (a Hawaiian environmental organization). PUNA was concerned with the impact of Bt food on the utility of Bt sprayables for organic farmers and, the risks of escapes from the experimental acreage. BPPD responded to PUNA with a letter addressing their concerns and indicating that EPA is also concerned about preserving the effectiveness of sprayable Bt products for organic farmers and is working to develop effective insect resistant management strategies to that end. On May 11, 1999, the EUP was amended to remove the requirement that plant material remaining after harvest or returned to the plot must be chopped or disked and deep-plowed to soil depths greater than 6 to 8 inches. On June 18, 1999, the EUP was amended to switch acreage between different protocols in the program at the same sites. On January 27, 2000, the EUP was amended to permit the planting of an additional 55 acres in Puerto Rico. On February 4, 2000, the EUP was amended to permit the planting of an additional 5 acres in Hawaii. On March 31, 2000, the EUP was extended/amended to allow the planting of an additional 145 acres. On April 21, 2000, the EUP was extended/ amended to allow the planting of an additional 947 acres. This amendment/ extension of the EUP is effective from April 21, 2000 to March 31, 2001. Thirteen comments were received in reply to the Federal Register notice announcing receipt of this amendment/ extension. Comments raised concerns about the labeling of food resulting from Bt corn, food safety, pollen shed/drift contamination of adjacent organic crops, the development of resistance to foliar Bt, the impact of testing on the Hawaiian environment, the impact on Bt corn on farmers in Puerto Rico, and the impact to non-target insects. Based on the information submitted, no significant or irreversible hazards from Cry1F corn to non-target organisms were anticipated for the duration of this limited acreage program.

Bt Crops Reassessment Process

In order to link these Cry1F Bt corn registrations to the current Bt crops reassessment process that the Agency is undergoing to ensure that any new necessary modifications to the registration and data requirements that are determined for Bt crops during the reassessment are imposed for these products, an expiration date of September 30, 2001 for the Cry1F products was imposed to match the expiration date of the currently registered Bt corn products being evaluated in the reassessment

EPA is currently engaged in a comprehensive reassessment of the time-limited registrations for all existing B.t. corn and cotton plant-pesticides. This reassessment has been designed to assure that the decisions on the renewal of these registrations are based on the most current health and ecological data. Current registrations are set to expire September 30, 2001. As part of EPA's reassessment, the Agency will be decide whether to extend the registrations and whether to include any additional terms and conditions of such registrations for issues including insect resistance management, the protection of non-target organisms, and other measures necessary to ensure full public and environmental safety.

II. Science Assessment

The classifications that are found for each data submission are assigned by the EPA science reviewer and are an indication of the usefulness of the information contained in the documents and meets the intent of the test guidelines. A rating of "ACCEPTABLE" indicates the data is useful for risk assessment, is scientifically valid and has been satisfactorily performed according to accepted EPA guidelines or other justified criteria. A "SUPPLEMENTAL" rating indicates the data provide some information that can be useful for risk assessment. However, "SUPPLEMENTAL" studies may either have certain aspects not adequately described to be scientifically acceptable (SUPPLEMENTAL. UPGRADEABLE) or have not been done to fulfill a specific EPA guideline requirement. If a study is rated as "SUPPLEMENTAL. UPGRADEABLE," there is always an indication of what is lacking or what can be provided to change the rating to "ACCEPTABLE." If there is simply a "SUPPLEMENTAL" rating, the reviewer will often state that the study is not required by current EPA guidelines or does not need to be reclassified as "ACCEPTABLE." An "UNACCEPTABLE" rating indicates that the study was scientifically compromised and cannot be used for risk assessment purposes.

A. PRODUCT CHARACTERIZATION

Study	***Result***	***MRID #***
Quantitative ELISA analysis of Cry1F expression levels in maize MPS inbreds and hybrid lines 1360, 1365, 1366, and 1369. (Interim report)	Maize plants (hybrids) from two locations grown under standard agronomic practices of the Midwestern Corn Belt were analyzed by ELISA for Cry1F protein content. The youngest leaf of expanding whorls at the V9 stage were collected from five plants per entry. Values of Cry1F protein for all four hybrids were similar, ranging from 1.52 to 2.63 pg/µg dry weight. Control hybrid A_M was negative for Cry1F as determined by ELISA. Classification: Acceptable	447148-04

Study	***Result***	***MRID #***
Product characterization data for *Bacillus thuringiensis* var. *aizawai* Cry1F as expressed in maize.	A modified (synthetic, less than full-length) form of the *cry*1Fa2 gene and the phosphinothricin acetyl transferase (*pat*) gene were inserted into maize plants by microprojectile bombardment. Three transformation events resulting from microprojectile bombardment will be evaluated under the proposed EUP: TC 1360, TC 1362 and TC 1507. Plants were analyzed for Cry1F by ELISA and PAT by application of glufosinate herbicide. Using a chi square analysis with a 95 % confidence interval, the expected Mendelian ratio of 1:1 was observed for both first and second generations for five inbreds with one exception; first generation TC 1632. Event TC 1507 has been analyzed for only the first generation and ratios (1:1) were as expected. Classification: Supplementary. The registrant should clarify the source of the ubiquitin exon and intron as being from the ubiquitin gene and not the promoter region. A determination of expression of the ubiquitin exon sequence is also needed and whether it alters the sequence of Cry1F.	447148-01

Supplement to MRID 447148-01: Supplemental Data – Product Characterization Data for *Bacillus thuringiensis* var. *aizawai* Cry1F Insect Control Protein as Expressed in Maize	This submission represents a clarification of nomenclature as presented in a previous submission and review. Labeling (in a previous submission) of the Ubi DNA fragment on the plasmid map should have indicated that it includes the Ubi ZM promoter and the first exon and intron of the Ubi ZM gene. The Ubi exon and intron are included in this construct (PHI8999), however, they have no effect on the structure of the Cry1F product, only on the expression of the gene. Exon 1 contains no ATG start site for translation. A translation initiation sequence (Kozak consensus sequence) situated just upstream from the start site (first translated ATG) drives translation of the mature, spliced mRNA. Classification: Acceptable.	450201-17
Characterization of gene inserts – *Bacillus thuringiensis* var. *aizawai* Cry1F insect control protein as expressed in maize.	The integration pattern of *cry*1F and *pat* genes introduced into event TC 1360 was analyzed by Southern blotting. Within the Southern analysis, two types of digests are employed to determine the complexity of DNA integration into the maize genome and to determine the copy number of integrated transgenes. Analysis of four of the progeny from event TC 1360 revealed the presence of two bands hybridizing to the *cry*1F probe; both bands appeared to hybridize with similar intensity. Hybridization to internal controls on the blot gave an indication of single copy integration and certainly no more than two copies of the insert integrated into the maize genome. When control plant DNA was probed, no hybridization was noted. TC 1360 and control DNA probed with the *kan*^{r} gene indicated no hybridization within these samples. Classification: Acceptable.	447148-02
Characterization of inserted genes in Cry1F maize line 1507	A modified (synthetic, less than full-length) form of the *cry1F* gene and the phosphinothricin acetyl transferase (*pat*) gene were inserted into maize plants by microprojectile bombardment. Digestion of the genomic DNA of maize line 1507 with *Nhe*I or *Hind*III and Southern hybridization with probes specific for *cry1F*, *kan*^{r} and *pat* genes yielded indications of the complexity of the gene integration pattern and copy number. Hybridization patterns suggested that the copy number of introduced / integrated *cry1F* and *pat* genes is one. It is most likely that the TC 1507 line contains one functional *cry1F* gene and partial copies (1 or 2) of the gene which are non-functional. It is not possible with this technique, however, to discern the functionality of probed sequences. No *kan*^{r} DNA was introduced into line 1507 during transformation, as indicated by the lack of signal when 1507 genomic DNA was probed with the *kan*^{r} gene. There was no hybridization signal when the non-transformed maize line 13-1 was probed with *pat* or *cry1F* or *kan*^{r}. Classification: Acceptable.	450201-02

Characterization of expressed Cry1F protein in maize tissues (pollen, grain, grain-containing feed, and purified maize-expressed Cry1F protein) and microbial expressed Cry1F delta endotoxin by biological and biochemical procedures.	Cry1F protein from maize 1507 pollen, grain, grain-derived feeds and a microbial source was evaluated biochemically using ELISA, SDS-PAGE and Western Blotting, and for bioactivity using insect bioassays. Control maize tissues were used to prepare comparable samples. Pollen from line 1507 contained Cry1F at 31 to 33 ng / mg pollen, while no Cry1F protein was detected in pollen from non-Cry1F plants. The purified maize-expressed Cry1F test substance was approximately 32 ng / mL extract. The comparable extract from non-Cry1F maize did not show any detectable Cry1F protein; the limit of detection (LOD) was 0.04 ng / mg sample. Coomassie stained gels indicated similar profiles for both control maize and Cry1F maize samples following SDS-PAGE. Antibodies directed against Cry1F detected this protein (64 kDa) in the Cry1F maize grain samples while there was no indication of any Cry1F protein in the control samples of grain. Pollen, maize-expressed Cry1F and microbially derived Cry1F were all active against the European Corn Borer larvae at the times tested. For the Tobacco Budworm larval bioassay, substances tested included maize grain, maize grain derived fish feed, and maize grain derived quail feed. Samples containing Cry1F maize grain and quail feed made from this grain had identical amounts of Cry1F protein based upon the GI_{50}s calculated. Comparison of control and Cry1F fish feed over four separate bioassays indicated that there was no statistical difference ($p = 0.05$) based upon ANOVA. Preparation of the fish feed sample reduced the biological activity of the Cry1F protein below sensitivity for the assay. Classification: Acceptable.	450201-03
Quantitative ELISA analysis of Cry1F and PAT expression levels in compositional analysis of maize inbred and hybrid lines 1362 and 1507	Protein expression values indicated substantial variability in protein levels for Cry1F in the tissues sampled. No definitive conclusions could be reached from the data presented when comparing levels of Cry1F in hybrid 1507 and inbred 1507 when examining pollen, silk, stalk, leaf, grain, whole plant and senescent whole plant samples. Since these hybrids and inbreds were grown in areas of Chile with similar climatic extremes to the maize growing areas of the U.S., it is anticipated that these values will represent those to be expected in the U.S. cornbelt. PAT expression was also not readily distinguishable when comparing inbred and hybrid expression values. The inability to detect PAT protein in the majority of samples, except leaf, is somewhat puzzling in that the plants demonstrated clear glufosinate tolerance at all field sites. Given the generally strong, non-tissue specific expression levels typically associated with the CaMV 35S promoter (driving *pat* expression), it is not readily apparent why more PAT protein was not detected in more samples. Its presence in leaf tissue was expected, however, the reason for the absence in many of these samples is less than clear. Classification: Acceptable.	**450201-04**

Quantitative ELISA Analysis of Cry1A(b) Expression Levels in and Compositional Analysis of Hybrid Lines Derived from Event 176	General agronomic performance, nutrient analysis and Cry1A(b) expression data suggest the equivalence of maize plants grown in South and North America and the feasibility of using winter grow out plots to extend the breeding season or evaluate further traits on either continent. Results from the compositional analysis for fatty acids, amino acids, and minerals for whole plant and grain samples demonstrated that the maize grown on either continent were comparable, however, values for amino acids in Chile were beyond the previously observed ranges for 7 of the 18 examined; with these amino acids, the percent differences ranged from 2 to 13 % higher. Given the typical biological variability observed in any field or natural situation, these differences, while above what was expected, are not substantially out of line with what was previously known. When total protein levels were measured between countries, there were some statistically different values. Except for the leaf expression data from Chile, in all cases the ranges overlapped between growing regions and the differences observed (1 to 2 fold in some cases) were not totally unexpected for a biological system grown in an environment with several variables (*e.g.*, water relations, GDU, soil type). Classification: Acceptable.	451311-04
Phosphinothricin acetyltransferase (PAT) protein: In Vitro Digestibility Study	Stability of the PAT protein was examined using a simulated gastric fluid (pH 2.0) assay. PAT was observed to degrade within 5 seconds as judged by SDS-PAGE mobility. At 5 seconds, intermittent trace fragments were visible on the gel, but were gone by 10 seconds. The BSA (bovine serum albumin) positive control was digested by 1 min, as expected, while the β-lac (β- lactoglobulin) negative control remained undigested after 10 min. This finding indicates that the PAT protein is not stable in the gastric environment and degrades to smaller peptide fragments rapidly. Classification: Acceptable.	450415-01
Cry1F Lateral Flow Test Kit Procedure for Analyzing Cry1F Corn Grain	A double antibody sandwich test was developed to detect the Cry1F protein in homogenized maize grain samples using a rapid test method. A double antibody sandwich technique is used in the Lateral Flow Test Kit for Cry1F. Antibodies raised against the Cry1F protein are incorporated into the Lateral Flow test strip and coupled to a color reagent. When in contact with Cry1F protein, the antibodies bind Cry1F and a sandwich is formed, however, not all of the antibodies are coupled to the color reagent. The test strips contain two zones wherein capture of color reagent or antibodies can occur. One zone captures bound Cry1F and the other captures color reagent. Both zones display a reddish color when protein-antibody sandwich and / or unreacted color reagent are captured. When only one line (control) line is present, a negative sample is indicated, while the presence of two lines indicates the presence of Cry1F. The Cry1F Lateral Flow Test Kit accurately detected Cry1F protein in 30 of 30 corn kernels from Cry1F maize and indicated negative reactions for the 30 control maize kernels. This finding demonstrates the utility of using the Cry1F Lateral Flow Test Kit for detection of Cry1F protein in maize grain samples. This kit allows for a rapid qualitative determination of the presence of Cry1F protein. Classification: Acceptable.	452793-01

Method Validation Report for the Determination of Cry1F Delta-endotoxin Protein in Corn Grain by Enzyme Linked Immunosorbent Assay	The results of this assay validation indicate that the ELISA based assay was suitable for the analysis of Cry1F as found in maize grain. Average recoveries from samples spiked with Cry1F protein (truncated microbial form) were between 67 and 107 %. Extractions from known Cry1F maize grain samples demonstrated that a sample as small as 50 mg could be properly extracted and quantified. Classification: Acceptable.	452793-02
Thermolability of Cry1F (truncated) Delta-Endotoxin	The Cry1F test substance was prepared in 10 mM potassium phosphate buffer (pH 7.5) and placed into a water bath at either 60, 75 or 90 EC for 30 minutes, or into the refrigerator at 4 EC. Application of treated Cry1F to the surface of an insect diet and measurement of growth inhibition of neonate tobacco budworm larvae, indicated that the Cry1F protein was labile to heat at and above 75 EC. Classification: Acceptable.	452748-01
Compositional Analysis of Maize MPS Hybrid Line 1507	Protein and nutritional parameters were measured in grain and whole plant samples of hybrid 1507 (expressing Cry1F) and a genetically similar control hybrid, both grown at 4 locations in Chile. Fatty acids, ash, vitamins, fiber, moisture, amino acids, minerals and antinutrients were examined using standard tests. No difference was observed between levels of these constituents in the hybrid 1507 when compared to commercial hybrids not encoding this gene, however, the non-essential amino acid, glutamic acid, was slightly above the known ranges for both the control and test lines. Classification: Acceptable.	452748-02
Equivalency of microbial and maize expressed Cry1F protein; Characterization of test substances for biochemical and toxicological studies.	Standard techniques of protein chemistry were used to assess similarities between the bacterial and plant sources of the Cry1F protein. Additionally, insect mortality assays were performed to determine *in vitro* toxicity. An *in vitro* digestibility assay was done to determine that Cry1F was unstable under conditions simulating the gastric environment. This simulation of gastric conditions indicated that the toxin (from microbial source) was readily digested by pepsin. SDS-PAGE and Western blotting of plant and bacterial sources determined the presence of a 65 kDa protein corresponding to the trypsinized core of the δ-endotoxin. Plant extracts contained 0.158 % Cry1F as determined by ELISA; control plants were negative. N-terminal sequencing of 5 aa determined that the microbial and plant expressed protein maintained this sequence intact. Glycosylation was not evident in Cry1F from either source. Classification: Acceptable.	447149-03

B. HUMAN HEALTH ASSESSMENT

I. Mammalian Toxicity and Allergenicity Assessment

Data have been submitted demonstrating the lack of mammalian toxicity at high levels of exposure to the pure Cry1F protein. These data demonstrate the safety of the products at levels well above maximum possible exposure levels that are reasonably anticipated in the crops. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived. [See 40 CFR Sec. 158.740(b)(2)(i).] For microbial products, further toxicity testing and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study, to verify the observed effects and clarify the source of these effects (Tiers II & III).

The acute oral toxicity data submitted support the prediction that the Cry1F protein would be non-toxic to humans. Male and female mice (5 of each) were dosed with 15 % (w/v) of the test substance, which consisted of *Bacillus thuringiensis* var. *aizawai* Cry1F protein at a net concentration of 11.4 %. Two doses were administered approximately an hour apart to achieve the dose totaling 33.7 mL / kg body weight. Outward clinical signs and body weights were observed and recorded throughout the 14 day study. Gross necropsies performed at the end of the study indicated no findings of toxicity. No mortality or clinical signs were noted during the study. An LD_{50} was estimated at >5050 mg / kg body weight of this microbially produced test material. The actual dose administered contained 576 mg Cry1F protein / kg body weight. At this dose, no LD_{50} was demonstrated as no toxicity was observed. Cry1F maize seeds contain 0.0017 to 0.0034 mg of Cry1F / gram of corn kernel tissue.

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjoblad, Roy D., *et al*. "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Therefore, since no effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the Cry1F protein is not considered toxic. Further, amino acid sequence comparisons showed no similarity between Cry1F protein to known toxic proteins available in public protein databases.

Since Cry1F is a protein, allergenic sensitivities were considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, may be glycosylated and present at high concentrations in the food.

Data has been submitted which demonstrates that the Cry1F protein is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. In a solution of Cry1F:pepsin at a molar ratio of 1:100, complete degradation of Cry1F to amino acids and small peptides occurred in 5 minutes. A heat lability study demonstrated the loss of bioactivity of Cry1F protein to neonate tobacco budworm larvae after 30 minutes at 75 EC. Studies submitted to EPA done in laboratory animals have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the δ-endotoxin of the crystal protein. Additionally, a comparison of amino acid sequences of known allergens uncovered no evidence of any homology with Cry1F, even at the level of 8 contiguous amino acids residues.

The potential for the Cry1F protein to be a food allergen is minimal. Regarding toxicity to the immune system, the acute oral toxicity data submitted support the prediction that the Cry1F protein would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjoblad, Roy D., et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Therefore, since no effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the Cry1F protein is not considered toxic.]

II. Aggregate Exposures

Pursuant to FFDCA section 408(b)(2)(D)(vi), EPA considers available information concerning aggregate exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations include dietary exposure under the tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectant chemical residue, and exposure from non-occupational sources. Exposure via the skin or inhalation is not likely since the plant-incorporated protectant is contained within plant cells, which essentially eliminates these exposure routes or reduces these exposure routes to negligible. Oral exposure, at very low levels, may occur from ingestion of processed corn products and, potentially, drinking water. However a lack of mammalian toxicity and the digestibility of the plant-incorporated protectants have been demonstrated. The use sites for the Cry1F protein are all agricultural for control of insects. Therefore, exposure via residential or lawn use to infants and children is not expected. Even if negligible exposure should occur, the Agency concludes that such exposure would present no risk due to the lack of toxicity demonstrated for the Cry1F protein.

III. Cumulative Effects

Pursuant to FFDCA Section 408(b)(2)(D)(v), EPA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Because there is no indication of mammalian toxicity to these plant-incorporated protectants, we conclude that there are no cumulative effects for the Cry1F protein.

IV. Determination of Safety for U.S. Population, Infants and Children

A) Toxicity and Allergenicity Conclusions

The data submitted and cited regarding potential health effects for the Cry1F protein include the characterization of the expressed Cry1F protein in corn, as well as the acute oral toxicity, heat stability, and *in vitro* digestibility of the proteins. The results of these studies were determined applicable to evaluate human risk and the validity, completeness, and reliability of the available data from the studies were considered.

Adequate information was submitted to show that the Cry1F test material derived from microbial cultures was biochemically and, functionally similar to the protein produced by the plant-

incorporated protectant ingredients in corn. Production of microbially produced protein was chosen in order to obtain sufficient material for testing.

The acute oral toxicity data submitted supports the prediction that the Cry1F protein would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels [Sjoblad, Roy D., et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)]. Since no effects were shown to be caused by Cry1F protein, even at relatively high dose levels (>5,050 mg test substance / kg body weight; 576 mg Cry1F / kg body weight), the Cry1F protein is not considered toxic. This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived. [See 40 CFR Sec. 158.740(b)(2)(i).] For microbial products, further toxicity testing and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study to verify the observed effects and clarify the source of these effects (Tiers II & III).

Although Cry1F expression level data was required for an environmental fate and effects assessment, residue chemistry data were not required for a human health effects assessment of the subject plant-incorporated protectant ingredients because of the lack of mammalian toxicity.

Both (1) available information concerning the dietary consumption patterns of consumers (and major identifiable subgroups of consumers including infants and children); and (2) safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food additives, are generally recognized as appropriate for the use of animal experimentation data were not evaluated. The lack of mammalian toxicity at high levels of exposure to the Cry1F protein demonstrates the safety of the product at levels well above possible maximum exposure levels anticipated in the crop.

The genetic material necessary for the production of the plant-incorporated protectants active ingredients are the nucleic acids (DNA, RNA) which comprise (1) genetic material encoding these proteins and (2) their regulatory regions. "Regulatory regions" are the genetic material, such as promoters, terminators, and enhancers, that control the expression of the genetic material encoding the proteins. DNA and RNA are common to all forms of plant and animal life and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to their consumption as a component of food. These ubiquitous nucleic acids, as they appear in the subject active ingredient, have been adequately characterized by the applicant. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the subject active plant pesticidal ingredients.

B) Infants and Children Risk Conclusions

FFDCA section 408(b)(2)(C) provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues and the cumulative effects on infants and children of the residues and other substances with a common mechanism of toxicity. In addition, FFDCA section 408(B)(2)(C)

also provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database unless EPA determines that a different margin of safety will be safe for infants and children.

In this instance, based on all the available information, the Agency concludes that there is a finding of no toxicity for the Cry1F protein and the genetic material necessary for its production. Thus, there are no threshold effects of concern and, as a result, the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply.

C) Overall Safety Conclusion

There is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry1F protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

The Agency has arrived at this conclusion because, as discussed above, no toxicity to mammals has been observed for the plant-incorporated protectants.

V. Other Considerations

A) Endocrine Disruptors

The pesticidal active ingredients are proteins, derived from sources that are not known to exert an influence on the endocrine system. Therefore, the Agency is not requiring information on the endocrine effects of these plant-pesticides at this time.

B) Analytical Method(s)

A validated method for extraction and direct ELISA analysis of Cry1F in corn grain has been submitted and found acceptable by the Agency.

C) Codex Maximum Residue Level

No Codex maximum residue levels exists for the plant-incorporated protectants *Bacillus thuringiensis* Cry1F protein and the genetic material necessary for its production in corn.

VI. Tolerance Exemption

Therefore, 40 CFR chapter I was amended to add section 180.1217.

Section 180.1217 *Bacillus thuringiensis* Cry1F Protein and the Genetic Material Necessary for its Production in Corn; exemption from the requirement of a tolerance.

Bacillus thuringiensis Cry1F protein and the genetic material necessary for its production in corn are exempt from the requirement of a tolerance when used as plant-pesticides (now known as plant-incorporated protectants) in the food and feed commodities of field corn, sweet corn and popcorn. ``Genetic material necessary for its production'' means the genetic material which comprise: genetic material encoding the Cry1F protein and its regulatory regions. ``Regulatory regions'' are the genetic material, such as promoters, terminators, and enhancers, that control the expression of the genetic material encoding the Cry1F protein.

VII. Supporting Data

Study	Result	MRID #
Acute oral toxicity study in mice: Cry1F *Bacillus thuringiensis* var. *aizawai* delta-endotoxin.	Dosing of ten albino mice with bacterial cell protein containing the d-endotoxin of *Bacillus thuringiensis* var. *aizawai* at > 5050 mg/kg (0.576 g/kg of Cry1F) body weight resulted in no mortality and no observed gross abnormalities. All animals appeared normal during the study and all except one gained weight throughout the study. Classification: Acceptable. Toxicity category III based on dose given with no observable effect.	446911-01
Supplement to MRID 446911-01: Supplemental Data for Acute Oral Toxicity Study in Mice: Cry1F *Bacillus thuringiensis* var. *aizawai* delta-endotoxin	This submission represents a clarification of test substance as presented in a previous submission and review. The acute oral toxicity study dosed mice at > 5050 mg microbial protein / kg body weight. The actual dose administered contained 576 mg Cry1F protein / kg body weight. At this dose, no LD_{50} was demonstrated as no toxicity was observed. The truncated form of the protein represents amino acids 28-612 of the Cry1F toxin sequence, whereas the plant-expressed form of Cry1F contains amino acids 1-605. The truncated form used in the oral toxicity study adequately represents that toxin to be found in the plant expression system. Classification: Acceptable.	450201-18

Comparison of amino acid sequence similarity of Cry1F and PAT proteins to known allergen proteins	A modified (synthetic) form of the *cry*1Fa2 gene and the phosphinothricin acetyl transferase (*pat*) gene were inserted into maize plants by microprojectile bombardment. A database of available sequenced allergens and toxins was searched for similarity to both the less than full-length Cry1F and PAT proteins such that a level of eight, contiguous amino acid homology would be detected. This number of contiguous amino acids is considered to be the smallest antigenic portion of a protein (peptide) to induce an allergic reaction based upon T-cell recognition in a sensitized individual. The database search and comparison to known allergens from plant, bacterial, fungal and animal origins indicates that no significant amino acid homology exists for Cry1F or PAT with any of these proteins. For both proteins of interest, the lack of any significant amino acid homology indicates that the potential for an immunological response developing into a food allergy from consumption of these proteins is low. Classification: Acceptable.	449717-01
Equivalency of microbial and maize expressed Cry1F protein; Characterization of test substances for biochemical and toxicological studies.	Standard techniques of protein chemistry were used to assess similarities between the bacterial and plant sources of the Cry1F protein. Additionally, insect mortality assays were performed to determine *in vitro* toxicity. An *in vitro* digestibility assay was done to determine that Cry1F was unstable under conditions simulating the gastric environment. This simulation of gastric conditions indicated that the toxin (from microbial source) was readily digested by pepsin. SDS-PAGE and Western blotting of plant and bacterial sources determined the presence of a 65 kDa protein corresponding to the trypsinized core of the δ-endotoxin. Plant extracts contained 0.158 % Cry1F as determined by ELISA; control plants were negative. N-terminal sequencing of 5 aa determined that the microbial and plant expressed protein maintained this sequence intact. Glycosylation was not evident in Cry1F from either source. Classification: Acceptable.	447149-03
Thermolability of Cry1F (truncated) Delta-Endotoxin	The Cry1F test substance was prepared in 10 mM potassium phosphate buffer (pH 7.5) and placed into a water bath at either 60, 75 or 90 EC for 30 minutes, or into the refrigerator at 4 EC. Application of treated Cry1F to the surface of an insect diet and measurement of growth inhibition of neonate tobacco budworm larvae, indicated that the Cry1F protein was labile to heat at and above 75 EC. Classification: Acceptable.	452748-01

C. ENVIRONMENTAL ASSESSMENT

1. Ecological Effects Hazard Assessment

This environment hazard assessment includes outcrossing and potential for weeds to develop if pollen from Cry1F corn was to fertilize other plants, horizontal gene transfer, expression of Cry1F protein in plant tissues, ecological effects including effects on monarch butterflies, fate of Bt proteins in the environment and effects on endangered species, particularly Lepidoptera. Studies

have been submitted which demonstrate no effects under test conditions to representative species of birds (Bobwhite quail), non-target soil organisms (*Collembola* and Earthworm), honey bees, ladybird beetle, green lacewing, parasitic wasp, the monarch butterfly, aquatic invertebrates (*Daphnia magna*) and non-target insects in corn fields. In addition, it has been shown that conventional processes used in the commercial preparation of fish food inactivate any Cry1F protein present in corn grain. Cry1F protein in soil has been shown to degrade rapidly to very low levels. A listing of the submitted studies and the results is found in Table 1 below.

Table # 1: Non-Target Toxicity Studies

Guideline No.	Study	Result (LC_{50})	Status, Classification & Comments	MRID #
OPPTS Series 885-4380	Evaluation of the Dietary Effect(s) on Honeybee (*Apis mellifera*) Development Using Bacterially Expressed Bt Cry1F Delta-Endotoxin and Pollen from Maize Expressing Bt Cry1F Delta Endotoxin.	LC_{50} > 64 ng Cry1F in 2 mg pollen /larva and 640 ng Cry1F protein /larva	The data show that at the expected environmental exposure the proposed use of Cry1F protein in corn is not likely to have any measurable deleterious effects on the honey bee (*Apis mellifera*). The study is scientifically sound and no treatment mortality or behavior change was observed between the dosed and control replicates. Cry1F protein as expressed in corn pollen showed no toxicity to honey bee larvae or their development into healthy adults. The test insects were exposed to a constant dose of pollen. This is more than the amount that the bees would be expected to consume under field use conditions. As a result, no discernible detrimental effects to honey bees are expected from the proposed uses of the Cry1F producing corn. This study adequately address potential toxicity concerns for honey bees exposed to Cry1F protein expressed in corn pollen in the field. Classification: Acceptable.	450415-03, 453078-05 (supplement)
71-2, 154-7	Transgenic Corn Expressing *Bacillus thuringiensis* var. *aizawai* (Bt) Cry1F Delta-Endotoxin: A Dietary Toxicity Study with the Northern Bobwhite.	LC_{50} > 100,000 ppm (10% corn meal); NOEC > 100,000 ppm. (10% corn meal)	The dietary LC_{50} value for corn grain (meal) expressing *Bacillus thuringiensis* var. *aizawai* protein in corn grain when fed to juvenile northern bobwhite for 5 days was determined to be greater than 100,000 ppm (10% corn meal), the only concentration tested. The no-observed-effect concentration was also 100,000 ppm. These data show that there will be no adverse effects on avian wildlife from incidental field exposure to Cry1F corn. These data are, however, not sufficient to make a hazard assessment from repeated exposure(s) to higher doses of Bt corn. A six week study with 60 to 70% corn in the diet is necessary to assess hazards from chronic exposure of wild and domesticated fowl. Classification: Supplemental due to the concentration of corn meal tested.	450201-12
72-2, 154-9	A 48-Hr Static Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*) Using Bacterially-Expressed Bt Cry 1F Delta-Endotoxin and Pollen from Maize Expressing Bt Cry 1F.	The 48-hr EC50 was > 100 mg a.i./L. The NOEC was >100 mg a.i./L.	This study was conducted according to approved EPA guideline procedures. The 48-hr EC50 for *Daphnia magna* exposed to Bt Cry 1F delta-endotoxin was > 100 mg a.i./L. The no-mortality concentration and NOEC were estimated to be >100 mg a.i./L. There were no overt signs of toxicity to daphnids exposed to 100 mg *Bt*-pollen/L - (maize pollen containing the *Bt* Cry1F delta-endotoxin). These data show that there will be no adverse effects on daphnia from incidental field exposure to transgenic corn pollen containing Cry1F. Classification: Acceptable.	450201-08
OPPTS Series 885-4200	Waiver Request: Fish Toxicity Test with Transgenic Maize (Corn) Containing *Bacillus thuringiensis* var. *aizawai* (Bt) Cry 1F Delta-Endotoxin.	N/A	The Agency previously has waived static renewal toxicity tests for freshwater fish due to the lack of substantial exposure to Cry protein in runoff and corn pollen. Therefore the registrant's request to waive rainbow trout and bluegill sunfish toxicity studies is granted. In addition, based on submitted results of a protein-specific ELISA analysis and bioasays, no Cry1F protein was detectable in catfish pellets processed from corn kernels containing Cry1F protein. The submitted data and results are sufficient to conclude that the Cry1F protein in aquafarm fish diets is unlikely to present hazardous exposures. Accordingly the registrant's request to waive farmed fish toxicity studies is granted.	450442-01

OPPTS Series 885-4340	Chronic Exposure of *Folsomia candida* to Bacterially Expressed Cry1F Protein.	LC50 and NOEL >12.5 mg Cry1F/kg soil	Three treatment levels (12.5, 3.1, 0.63 mg/kg) of Cry1F were replicated four times for a total of 12 replicates. Test or control diets and water were given to the Collembola every two or three days for 28 days. In addition to a test and control treatment, thiodicarb (carbamate) was used as a reference substance. The number of surviving Collembola in each treatment were counted every seven days and at the end of 28 days. It was estimated that the exposure rates in this study are 1560-, 388-, and 79-fold-higher than would be encountered in the field. Results of the study indicate that levels of Cry1F that might occur in the field are not expected to adversely effect the soil invertebrate Collembola species. This study adequately addresses the Collembola environmental hazard issue. Classification: Acceptable.	450201-07
OECD Guideline 207	Cry 1F Bacillus thuringiensis var. aizawai delta Endotoxin: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate.	LC50 and NOEL >2.26 mg Cry1F/kg dry soil	There were no apparent treatment-related effects on mortality or body weight of worms in study. The NOEC value was determined to be equal to 1.7 mg Cry1F/kg dry soil and LC_{50} value was determined to be greater than the test concentration of 1.7 mg Cry1F/kg dry soil. [Actually 2.26 mg (Reviewer's comment: 33% moisture content appears to have been subtracted twice to obtain the 1.7 mg figure)] The 14-day LC_{50} value for earthworms exposed to chloroacetamide, a positive control, was determined to be approximately 15.7 mg a.i./kg dry soil . The one limit test concentration of 2.26 mg Cry1F/kg dry soil represented up to 100X the estimated concentration present in the top six inches of an acre of soil following the incorporation of 25,000 senescent corn plants. This concentration is higher than any amount of Cry protein that may be present in the soil during any stage of the growing season (such as from root exudation). Based on the results of this study, it is not likely that Cry1F transgenic corn plantings will have adverse effects on earthworms. Classification: Supplemental since test material analysis did not meet GLP standards.	450201-06, 453078-04 (supplement)
OPPTS Series 885.4340	Cry1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study With Green Lacewing Larvae.	LC_{50} and NOEC > 480 ppm a.i (pollen expressing 32 ng Cry1F/mg pollen)	Green lacewing larvae fed a concentration of Bt Cry1F protein at 15x the expected rate found in corn pollen (pollen expressing 32 ng Cry1F/mg pollen) resulted in no mortality or signs of toxicity or abnormal behavior over a 13 day period (>20% control mortality period). The LC_{50} and NOEC was determined to be >15x the concentration of Cry1F found in pollen and the was determined to be > 480 ppm a.i (the test concentration). Mortality and pupation rate were comparable between the treatment and control group. Therefore Cry1F at concentrations <15x that found in corn pollen should have no detectable adverse effects on *Chrysoperla carnea* in the field. This study adequately addresses the green lacewing environmental hazard issue. Classification: Acceptable.	450201-09, 453078-01 (supplement)
OPPTS Series 885.4340	Cry1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study With the Parasitic Hymenoptera. (*Nasonia vitripennis*)	LC_{50} and NOEC > 320 ppm a.i (pollen expressing 32 ng Cry1F/mg pollen)	Parasitic Hymenoptera fed a concentration of Bt Cry1F protein 10x the expected rate found in corn pollen (expressing 32 ng Cry1F/mg pollen) showed no mortality or signs of toxicity or abnormal appearance or behavior of surviving wasps in the treatment or control group over a 12 day period. The test was terminated after 12 days because 20% mortality was reached in the negative control. The NOEC and the LC_{50} were determined to be > 320 ppm a.i (10x field rate when calculated for pollen expressing 32 ng Cry1F/mg pollen). Therefore no hazard at field use rates is expected from the cultivation of Cry1F containing corn. This study adequately address potential concerns for Cry1F protein expressed in corn to parasitic Hymenopreta. Classification: Acceptable.	450201-11, 453078-03 (supplement)

OPPTS Series 885.4340	Cry1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study With the Ladybird Beetle. (*Hippodamia convergens*).	LC_{50} and NOEC > 480 ppm a.i (pollen expressing 32 ng Cry1F/mg pollen)	Adult lady beetles fed a concentration of Bt Cry1F protein at 15x the expected rate found in corn pollen (pollen expressing 32 ng Cry1F/mg pollen) resulted in no mortality or signs of toxicity over a 29 day period. Therefore, the NOEC and the LC_{50} were determined to be >15x the concentration of Cry1F found in pollen determined to be > 480 ppm a.i (the test concentration). The test insects were exposed to a dose of active ingredient approximating the amount that would be ingested by the beetles feeding on aphids under field conditions. As a result, no discernible beneficial beetle population effects are expected from the proposed uses of the Cry1F producing corn. This study adequately address potential concerns for Cry1F protein expressed in corn to beneficial beetles. Classification: Acceptable.	450201-10, 453078-02 (supplement)
OPPTS Series 885.4340	Toxicity of the Cry1F Protein to Neonate Larvae of the Monarch Butterfly (*Danaus plexippus* (Linnaeus).	LC_{50} > 10,000 ng/mL. NOEC <10,000 ng/mL.	First instar larval weight and mortality were recorded after seven days of feeding. There was no mortality to monarchs fed 10,000 ng/mL diet, the highest rate tested. There was some growth inhibition at 10,000 ng/mL diet. The study is scientifically sound. Since doses equivalent to 10,000 ng/mL diet are not likely to occur in nature, it can be concluded that Cry1F protein will not pose a risk to monarchs. Classification: Acceptable. RECOMMENDATIONS: The conclusions should be confirmed by providing data showing that the amounts of Cry protein found on milkweed leaves in the field are at concentrations less than the 10.000 ng/mL diet used in this study. The NOEC also has to be determined.	451311-02

154-35	Field Survey of Beneficial Arthropods Associated With *Bacillus thuringiensis* Cry1F Maize.	N/A	Sticky traps were set out weekly for six weeks. In addition, ten plants in the center row were visually evaluated for beneficial arthropods weekly for six weeks. Beneficial insects counted in this study were: lady beetles (*Cycloneda munda* & *Coleomegilla maculata*), predacious Carabids, brown lacewings (Hemerobiidae), green lacewings (*Chrysoperla plorabunda*), minute pirate bugs (*Orius insidiosus*), assassin bugs (Reduviidae), damsel bugs (Nabidae), Ichneumonid and Braconids (parasitic wasps), damselflies and dragonflies, and spiders. Data included counts of adult and larval lady beetles and lacewings when appreciable numbers of were collected. Visual counts showed no significant differences between the number of arthropods collected in Bt Cry1F corn and the non-transgenic isolines with two exceptions. There was a significantly greater number of lady beetles in the 1507 corn line and a significantly greater number of spiders in the 1360 line than the non-transgenic isolines. For lady beetles, there were an average of 1.2 beetles in the 1507 line compared to 0.6 beetles in the non-transgenic line. For spiders, the 1360 line averaged 1.8 spiders per ten plants and the non-transgenic isoline averaged 0.5 spiders per ten plants. In addition, significantly more *Orius* were found in the 1507 line then the non-transgenic line on two of the three sample dates. In general, the most beneficial insects found by visual counts were in the 1507 Cry1F corn line. Sticky trap counts showed that no significant differences between the number of most arthropod species in the transgenic corn and their respective isolines. However, the average number of parasitic Hymenoptera and *Orius* observed across all sample dates were significantly greater in the 1507 line then the non-transgenic isoline. For parasitic Hymenoptera, there was an average of 1.7 wasps per trap in the 1507 line compared to the average of 1 per trap in the non-transgenic isoline. For all arthropods, the average number of insects per trap in the non-transgenic 1507 isoline was 11.1 and the average number in the transgenic 1507 line was 8.7. The non-transgenic 1360 isoline averaged 9.5 insects per sticky trap while an average of 6.8 insects per sticky trap were collected in the 1360 transgenic line. The study is scientifically sound. There was no consistent pattern of differences in abundance of predatory insects on the Cry1F versus the control corn plots during the sampling period. This field census study adequately address potential concerns for Cry1F protein expressed in corn to non-target insect populations. Classification: Acceptable. RECOMMENDATIONS: Testing of larger plot sizes would, however, produce more significant results. Therefore it is recommended that the beneficial insect monitoring should continue into the first few years of commercial use of Cry1F corn crops to confirm the single season small plot “no effects” findings and make long range observations on non-target insect effects and abundance.	450201-13

N/A	Non-target Exposure and Risk Assessment for Environmental Dispersal of Cry1F Maize Pollen. (A probabilistic risk assessment)	N/A	To consider the exposure of non-target species including endangered Lepidoptera species to field corn pollen expressing the Cry1F delta endotoxin by evaluating pollen dissemination. The Cry1F concentration found in pollen occurring on milkweeds near the edge of Bt corn fields was predicted. Distance of pollen dispersal, levels of Cry1F expression in pollen, milkweed distribution and biomass from the edge of the field, pollen grain physical properties, and spatial-temporal availability of Cry1F to monarch larvae was determined. According to a probability-log plot demonstrating lepidopteran species susceptibility to Cry1F, 99% of lepidopteran species exhibit an LC_{50} of $0.06 μg g^{-1} which is 290-fold lower than the geometric mean LC_{50} (12.4 μg g^{-1}) and lower than the most sensitive lepidopteran species. The toxicity threshold, or no effect level for monarch neonates, for the Tier 1 risk assessment was determined to be 10 μg g^{-1} diet. When fed up to 10 μg g^{-1} Cry1F microbial toxin in diet, neonate monarch larvae were not affected. The toxicity threshold, or no effect level for monarch neonates, for the Tier 1 risk assessment was determined to be 10 μg g^{-1}. The log-probability plot of the Bt LC_{50} for lepidopteran species shows that the EEC does not exceed the LC_{50} for 98% of the intergenera population beyond 1 m from the field edge. The LC_{50} is not exceeded for 90% of the population 0.2 m from the edge. For monarch larvae, the no effect level (10 μg g^{-1}) occurs near the 50^{th} percentile intergenera LC_{50}. Since there is a rapid fall-off in exposure with distance, there is limited potential for non-target effects beyond the immediate field border. In addition, the estimated risk quotients (ratio of exposure to effect) demonstrate a lack of concern for monarchs (or other lepidopteran species) beyond 1 m from the field edge. The RQ in the corn field is 0.096. Classification: Acceptable.	450415-02

2. Outcrossing and Weediness

The movement of transgenes from the host plant into weeds and other crops has been a significant concern due to the possibility of novel exposures to the pesticidal substance. The Agency has determined that there is no significant risk of gene capture and expression of Cry1F protein by wild or weedy relatives of corn in the U.S., its possessions or territories. Domesticated corn does not have a reasonable possibility of passing its traits to wild maize species. Feral species related to corn, as found within the United States, cannot be pollinated due to differences in chromosome number, phenology (periodicity or timing of events within an organism's life cycle as related to climate, e.g., flowering time) and habitat.

However, concern over species related to maize (*Zea mays* ssp. *mays*), such as *Tripsacum* species and the teosintes, as potential recipients of gene flow from genetically modified *Zea mays* calls for a closer look at this topic. Some *Zea* spp., such as the teosintes, are known to be interfertile with maize and are discussed as potential recipients of pollen directed gene flow from maize. This issue is of particular concern based upon the increased planting of genetically modified maize.

a) *Zea mays* ssp. *mays* - Maize

The origin of corn is thought to be in Mexico or Central America, based largely on archaeological evidence of early cob-like maize in indigenous cultures approximately 7200 years ago. *Zea mays* is a wind-pollinated, monoecious, annual species with imperfect flowers. This means that spatially separate tassels (male flowers) and silks (female flowers) are found on the same plant, a feature

which limits inbreeding. A large variety of types are known to exist (*e.g.*, dent, flint, flour, pop, sweet) and have been selected for specific seed characteristics through standard breeding techniques. Maize cultivars and landraces are known to be diploid (2n = 20) and interfertile to a large degree. However, some evidence for genetic incompatibility exists within the species (*e.g.*, popcorn x dent crosses; Mexican maize landraces x Chalco teosinte). *Zea mays* has been domesticated for its current use by selection of key agronomic characters, such as a non-shattering rachis, grain yield and resistance to pests.

A recent study has indicated that cross-pollination of commercial maize cultivars at 100 ft downwind from the source of genetically modified maize was 1 %, and this proportion declined exponentially to 0.1 % at 130 ft and further declined to 0.03 % at 160 ft. At 1000 ft, the farthest distance measured, no cross-pollination was detected. For production of Foundation Seed, a distance of 660 ft has been generally required to ensure separation of pollen types. The relatively large size of corn pollen and its short viability period under most conditions preclude long distance transfer for purposes of outcrossing. Under conditions of high temperature or low humidity, corn pollen may only survive for a matter of minutes. Under more favorable conditions in the field or with controlled handling in the laboratory, pollen life may be extended to several hours.

b) *Tripsacum* species - Gama Grass

A close relative of corn or maize is the genus *Tripsacum.* Sixteen species of *Tripsacum* are known worldwide and generally recognized by taxonomists and agrostologists; most of the 16 different *Tripsacum* species recognized are native to Mexico, Central and South America, but three occur within the U.S.. In the Manual of Grasses of the United States, A. S. Hitchcock (revisions by Agnes Chase; 1971) reports the presence of three species of *Tripsacum* in the continental United States: *T. dactyloides*, *T. floridanum* and *T. lanceolatum*. Of these, *T. dactyloides*, Eastern Gama Grass, is the only species of widespread occurrence and of any agricultural importance. It is commonly grown as a forage grass and has been the subject of some agronomic improvement (*i.e.*, selection and classical breeding). *T. floridanum* is known from southern Florida and *T. lanceolatum* is present in the Mule Mountains of Arizona and possibly southern New Mexico.

For the species occurring in the United States, *T. floridanum* has a diploid chromosome number of 2n = 36 and is native to Southern Florida; *T. dactyloides* includes 2n = 36 forms which are native to the central and western U.S., and 2n = 72 forms which extend along the Eastern seaboard and along the Gulf Coast from Florida to Texas, but which have also been found in IL and KS; these latter forms may represent tetraploids (x = 9 or 18); and *T. lanceolatum* (2n = 72) which occurs in the Southwestern U.S. *Tripsacum* differs from corn in many respects, including chromosome number (*T. dactyloides* n = 18; *Zea mays* n = 10). Many species of *Tripsacum* can cross with *Zea*, or at least some accessions of each species can cross, but only with difficulty and the resulting hybrids are primarily male and female sterile. *Tripsacum* / maize hybrids have not been observed in the field, but have been accomplished in the laboratory using special techniques under highly controlled conditions.

Eastern Gama Grass is considered by some to be an ancestor of *Zea mays* or cultivated maize, while others dispute this, based largely on the disparity in chromosome number between the two species (maize n = 10; Gama Grass x = 9 or 18, with diploid, triploid and tetraploid races existing; 2n = 36 or 72), as well as radically different phenotypic appearance. Albeit with some difficulty, hybrids between the two species have been made. In most cases these progeny have been sterile or viable only by culturing with *in vitro* 'embryo rescue' techniques.

Even though some *Tripsacum* species occur in areas where maize is cultivated, gene introgression from maize under natural conditions is highly unlikely, if not impossible. Hybrids of *Tripsacum* species with *Zea mays* are difficult to obtain outside of the controlled conditions of laboratory and greenhouse. Seed obtained from such crosses are often sterile or progeny have greatly reduced fertility. Approximately 10 - 20% of maize-*Tripsacum* hybrids will set seed when backcrossed to maize, and none are able to withstand even the mildest winters. The only known case of a naturally occurring *Zea* - *Tripsacum* hybrid is a species native to Guatemala known as *Tripsacum andersonii*. It is 100% male and nearly 99% female sterile and is thought to have arisen from an outcrossing to teosinte, but the lineage is uncertain. *Zea mays* is not known to harbor properties that indicate it has weedy potential and, other than occasional volunteer plants in the previous season's corn field, maize is not considered as a weed in the U.S.

Relatively few accessions of *T. dactyloides* will cross with maize and the majority of progeny aren't fertile or viable even in those that do. In controlled crosses, if the female parent is maize, there is a greater likelihood of obtaining viable seed. When these hybrids have been backcrossed to maize in attempts to introgress *Tripsacum* genes for quality enhancement or disease resistance, the *Tripsacum* chromosomes are typically lost in successive generations. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the chromosomal complements of one of the parent species in subsequent generations.

Conclusion: The possibility of maize contributing genetic material to Eastern Gama Grass through random pollen flow in agricultural or natural situations is extremely remote based upon experience trying to create hybrids under the optimal laboratory conditions. No other known grass species present in the continental U.S. would interbreed with commercial maize populations (*i.e.*, be recipients of pollen-directed gene flow). None of the sexually compatible relatives of corn in the U.S. are considered to be serious, principal, or common weeds in the U.S.

c) *Zea* species - Teosintes

Teosintes, specifically *Z. mays* ssp. *mexicana* (Schrader) Iltis, *Z. mays* ssp. *parviglumis* Iltis and Doebley*, Z. mays* ssp. *huehuetenangensis* (Iltis and Doebley) Doebley*, Z. luxurians* (Durieu and Ascherson) Bird*, Z. perennis* (Hitchc.) Reeves and Mangelsdorf and *Z. diploperennis* Iltis, Doebley and Guzman*,* have co-existed and co-evolved in close proximity to maize in the Americas over thousands of years, however, maize and teosinte maintain distinct genetic constitutions despite sporadic introgression.

The teosintes retain a reduced cob-like fruit/inflorescence that shatters more than cultivated maize, but still restricts the movement of seeds as compared to more widely dispersed weedy species. Hence, the dispersal of large numbers of seeds, as is typical of weeds, is not characteristic of teosintes or maize. In their native habitat, some teosintes have been observed to be spread by animals feeding on the plants. Teosintes and teosinte-maize hybrids do not survive even mild winters and could not propagate in the U.S. corn belt. Additionally, some types have strict day length requirements that preclude flowering within a normal season (*i.e*., they would be induced to flower in November or December) and, hence, seed production under our temperate climate.

Since both teosinte and *Tripsacum* are included in botanical gardens in the U.S., the possibility exists (although unlikely) that exchange of genes could occur between corn and its wild relatives. EPA is not aware, however, of any such case being reported in the United States. Gene exchange between cultivated corn and transformed corn would be similar to what naturally occurs at the present time within cultivated corn hybrids and landraces. Plant architecture and reproductive capacity of the intercrossed plants will be similar to normal corn, and the chance that a weedy type of corn will result from outcrossing with cultivated corn is extremely remote.

Like corn, *Zea mays* ssp. *mexicana* (annual teosinte) and *Zea diploperennis* (diploid perennial teosinte) have 10 pairs of chromosomes, are wind pollinated, and tend to outcross, but are highly variable species which are often genetically compatible and interfertile with corn, especially when maize acts as the female parent. *Zea perennis* (perennial teosinte) has 20 pairs of chromosomes and forms less stable hybrids with maize. Corn and compatible species of teosinte are capable of hybridization when in proximity to each other. In Mexico and Guatemala, teosintes exist as weeds around the margins of corn fields. The F1 hybrids have been found to vary in their fertility and vigor. Those that are fertile are capable of backcrossing to corn. A few isolated populations of annual and perennial teosinte were said to exist in Florida and Texas, respectively. The Florida populations were presumably an escape from previous use of *Z. mays* ssp. *mexicana* as a forage grass, but local botanists have not documented any natural populations of this species for approximately twenty-five years. No teosinte populations are reported to exist in the State of Texas. Further, given the day length characteristics of *Z. diploperennis*, it is highly unlikely a sustaining population would result from introduction of this species. *Z. mays* ssp. *mexicana, Z. mays* ssp. *parviglumis, Z. luxurians* and *Z. diploperennis* may cross with maize to produce fertile hybrids in many instances. None of these teosinte species have, however, been shown to be aggressive weeds in their native or introduced habitats. Except for special plantings as noted above, teosinte is not present in the U.S. or its territories. Its natural distribution is limited to Mexico, Honduras, Nicaragua, El Salvador and Guatemala.

Given the cultural and biological relationships of various teosinte species and cultivated maize over the previous two millennia, it would appear that significant gene exchange has occurred (based upon morphological characters) between these two groups of plants and that no weedy types have successfully evolved as a result. More recent cytogenetic, biochemical and molecular analyses have indicated that the degree of gene exchange is far less than previously thought. Partial and complete gametophytic incompatibility has been documented among cultivated maize, landraces and teosinte . The former is demonstrated by differential pollen growth and a skewed recovery of alleles linked to

incompatibility genes. Complete incompatibility mechanisms serve to isolate a species or subspecies and are evidenced as pollen exclusion or non-functioning of pollen types on certain genotypes. Attempts to cross six collections of *Zea mays* ssp. *mexicana* with U.S. maize cultivars (W22, W23) yielded no or few seeds in five of the six groups.

Conclusion: Based on the ability of maize to hybridize with some teosintes, the suggestion of previous genetic exchange amongst these species over centuries, and their general growth habits, any introgression of genes into wild teosinte from *Zea mays* is not considered to be a significant agricultural or environmental risk. The growth habits of teosintes are such that the potential for serious weedy propagation and development is not biologically plausible in the United States.

Summary:

The potential for pollen-directed gene flow from maize to Eastern Gama Grass is extremely remote. This is evidenced by the difficulty with which *Tripsacum dactyloides* x *Zea mays* hybrids are produced in structured breeding programs. Additionally, the genus does not represent any species considered as serious or pernicious weeds in the United States or its territories. Any introgression of genes into this species as a result of cross fertilization with genetically-modified maize is not expected to result in a species that is weedy or difficult to control. In many instances where hybridization has been directed between these two species, the resultant genome is lacking in most or all of the maize chromosomal complement in subsequent generations.

Many of the *Zea* species loosely referred to as "teosintes" will produce viable offspring when crossed with *Zea mays* ssp. *mays*. None of these plants are known to harbor weedy characteristics and none of the native teosinte species, subspecies or races are considered to be aggressive weeds in their native or introduced habitats. In fact, many are on the brink of extinction where they are indigenous and will be lost without human intervention (*i.e*., conservation measures). Further, none of the landraces or cultivated lines of *Zea mays* are considered to have weedy potential and are generally considered to be incapable of survival in the wild as a result of breeding practices (*i.e*., selection) during domestication of the crop.

3. Ecological Exposure and Risk Characterization

a. Ecological Exposure

1) Maximum Expression of Cry1F Protein in Various Corn Tissues

Cry1F protein from inbred and hybrid maize 1507 pollen, grain, grain-derived feeds and a microbial source was evaluated biochemically using ELISA, SDS-PAGE and Western Blotting, and for bioactivity using insect bioassays. Transgene expression was found throughout the different plant tissues across the growing season. The level of the Cry1F proteins was higher in tissues and in whole plants during vegetative growth through pollen shed and declined with plant senescence. PAT expression was found to be typically below the detection limit.

a) Cry1F and PAT protein expression in hybrid maize samples:

Test line grain samples contained an average Cry1F expression of 89.8 (71.2 to 114.8) pg / µg total protein. Leaf sample expression from Cry1F maize lines was 110.9 (56.6 to 148.9) pg / µg total protein. Pollen and silk samples yielded 135.5 (113.4 to 168.2) pg/µg total protein for pollen (31 to 33 ng/mg pollen) and 50.3 (26.8 to 79.8) pg / µg total protein for silk. The Cry1F expression for stalk samples was 550.0 (355.9 to 737.4) pg / µg total protein. For whole plant samples, the expression level averaged 1063.8 (803.2 to 1572.7) pg / µg total protein. In senescent whole plant samples the expression of Cry1F was 714.3 (622.2 to 845.3) pg / µg total protein. Of the leaf samples tested for PAT expression, the test line samples ranged from below the LOD to 40.8 pg / µg total protein. All of the following tissues were below the LOD for PAT: pollen, silk, stalk and grain from both test and control lines. Both whole plant samples and senescent whole plant samples were negative or below the LOD for PAT.

b) Cry1F and PAT protein expression in inbred maize samples:

Test line grain samples contained an average Cry1F expression of 112.2 (66.5 to 141.5) pg / µg total protein. Leaf sample expression from Cry1F maize lines was 169.5 (79.3 to 209.4) pg / µg total protein. Pollen and silk samples yielded 207.5 (186.3 to 231.1) pg/µg total protein for pollen and 58.9 (36.2 to 89.8) pg / µg total protein for silk. The Cry1F expression for stalk samples was 637.8 (480.5 to 849.0) pg / µg total protein. For whole plant samples, the expression level averaged 1357.8 (1283.5 to 1428.0) pg / µg total protein. In senescent whole plant samples the expression of Cry1F was 677.5 (470.5 to 968.3) pg / µg total protein. Of the leaf samples tested for PAT expression, the test line samples ranged from below the LOD to 58.2 pg / µg total protein. All of the following tissues were below the LOD for PAT: pollen, silk, stalk and grain from both test and control lines. Both whole plant samples and senescent whole plant samples were negative or below the LOD for PAT.

2) Half-Life and Estimated Environmental Concentration

Based on a bioassay with the tobacco budworm (*Heliothis virescens*), a target species, purified Cry1F proteins incorporated into test soils biodegraded with a half-life of approximately 3.13 days (Table 2). This half-life is very comparable with the 4-7 days in published reports for other Cry proteins. The study does not, however, adequately address the duration and the amount of residual Cry 1F protein in the soil.

Table# 2: Environmental Fate Studies

Guideline No.	Study	Result (Half-life)	Status, Classification & Comments	MRID #
155-18	Environmental Fate of Cry1F Protein Incorporated Into Soil	DT_{50} = 3.13 days	Based on a bioassay with the tobacco budworm (*Heliothis virescens*), purified Cry1F protein in a powder form (11.4% Cry1F by weight) incorporated into Drummer Ap loam soil biodegraded over a 28-day period with a half-life of 3.13 days. The authors state that Cry1F will degrade in the soil within 28 days (the duration of this test). The study is adequate for half life determination. However, it is rated as supplemental. This study contained some deficiencies that should be addressed by the registrant. 1. It was stated that there were occasions when less than 30 bioassay wells were infested with larvae. However, how often this occurred and how many wells were infested in these situations was not detailed. 2. It is also a concern that wells containing more than one larvae were not scored. Details regarding how often this occurred and why there was potential escape from one well to another were not included in the report. 3. From this submission, it is not possible to determine how many larvae were bioassayed per treatment. 4. The presence of microbial activity (most important for soil protein degradation) or the presence of organic matter was also not discussed. 5. Root expression data were not submitted and are needed. In addition, statistical analysis included a linear regression. However, the Day 28 data point was dropped from the analysis because the change in EC_{50} did not appear linear between Day 14 and Day 28. If the data were not linear then a linear regression may not be appropriate. The study should be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in the soil. Although the deficiencies listed above do not result in this submission being considered inadequate for half-life determination, they should be addressed for the study to be upgraded from supplemental to acceptable. Classification: Supplemental.	450201-05

Much of the Cry1F that will be exposed to the soil or soil organisms in the field consists of the protein in various corn tissues, e.g. incorporation of crop debris at the end of the growing season, pollen, or root tissue. Several published studies indicate that Cry proteins expressed in transgenic corn degrade more rapidly in the soil than purified Cry protein. Testing of purified protein degradation in the soil, therefore, may result in higher soil half-life than the degradation of plant incorporated Cry1F. Therefore addition of purified Cry protein is likely a more rigorous test of degradation rates than addition of Cry1F corn tissue. The reported 3.13 day half life of purified protein does, however, indicate that the Cry1F protein will be degraded rapidly in the soil to levels below those that could pose a hazard to non-target organisms.

RECOMMENDATIONS: There is no evidence to indicate that prolonged exposure to trace amounts of Cry protein in the soil affects non-target organisms. The submitted data do not, however, sufficiently address the issue of residual Cry protein accumulation in the soil. The soil degradation study should be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in agricultural soil. Also, the soil used in the study should be actual field soil containing the microbial flora normally found in the field. This will give a more accurate rate of degradation of the Cry protein in the agricultural environment because microbial populations in the rhizosphere are commonly 100 fold higher than in bulk soil. Bulk soil generally does not support populations of microorganisms as high as those in the rhizosphere or those in soils with high organic

content (plant residues). In addition, field soil high in organic content should result in lower (if any) soil binding of Cry proteins.

Estimated Environmental Concentration (EEC): The amounts of Cry1F protein in an acre of corn (if 25,000 corn plants/acre at harvest were left in the field) is approximately 20.5 g/acre. As a result the expected maximum environmental concentration (EEC) of Cry1F protein will be 23 micrograms /kg dry soil (15 cm deep). This does not include any additional Cry protein in the soil as a result of root exudation (if root exudation is shown to occur).

RECOMMENDATIONS: Data for Cry1F protein expression in plant roots and data on Cry protein exudation by roots should be submitted for review.

3) Effects on soil microbial flora

Limited published data do not indicate that Cry proteins have any measurable effect on microbial populations in the soil, even at levels much higher than expected from Cry1F Bt corn cultivation. Due to frequent fluctuations of organic and other inputs into agricultural soil, at any particular time, soil samples are likely to display radically different abundances and diversity of microorganisms. There is no evidence to suggest that the numerous processes mediated by soil microorganisms do not persist across the spectrum from undisturbed soil under native vegetation to intensively cultivated soil under continuous cropping and chemical treatments. Without better information regarding the range of what constitutes natural microbial communities or microbial communities in current agroecosystems, and the consequences of such changes, it is not possible to assign a significance to apparently minor changes in microbial populations when they do occur. Constant fluctuations of soil microbial communities are typical of most soil ecosystems.

Summary: The low concentration of Cry protein in the soil has not been shown to have any adverse effects on non-lepidopteran organisms. Sufficient evidence exists to suggest that adverse impacts of Cry proteins in the soil are not likely, although the levels of expression in the root should be determined to assure that unexpectedly high levels of root expression do not exist. The EEC of Cry1F from corn (23 µg/kg dry soil) is well below levels used in toxicity tests which were performed at multiples of the expected environmental concentration in the soil.

4) Horizontal Transfer of Trangenes to Plants and Soil Organisms

Microbial transformation with large concentrations of plant transgenes has only been accomplished at low frequencies and under artificial optimized conditions in the laboratory , and only where homology to existing DNA in the recipient bacteria occurs. Under conditions where homology does not occur, horizontal transfer has not been observed. Therefore, DNA transfer occurs rarely if at all from plants to bacteria. In addition, because homologous sequences already exist in soil bacteria (such as native soil *Bacillus thuringiensis*) horizontal transfer of the same sequences from plants, if it were to occur, would not constitute a new phenomenon. Bt species are generally common in soil, if not always abundant, and therefore various *cry* genes have been available for long periods of time for horizontal transfer from Bt to plants or other soil species. Similarly, antibiotic resistance genes

and promoter genes used in making Bt plants have long been present in the soil microorganisms and decaying plant material. Therefore the likelihood of an adverse impact or new horizontal gene transfer that is not already capable of taking place in the soil is extremely unlikely.

b. Risk Characterization for Terrestrial Animals

1) Avian

The dietary LC_{50} value for corn grain (meal) expressing *Bacillus thuringiensis* var. *aizawai* Cry1F protein in corn grain when fed to juvenile northern bobwhite for 5 days was determined to be greater than 100,000 ppm (10% corn meal). The no-observed-effect concentration was also 100,000 ppm. The study is scientifically sound and no treatment mortality or behavior change was observed between the dosed and control replicates. These data show that there will be no adverse effects on avian wildlife from incidental field exposure to Cry1F corn. The data are, however, not sufficient to make a hazard assessment from repeated exposure(s) to higher doses of Bt corn. The study is rated as supplemental because the concentration tested (10% corn in the diet) is too low to assess hazards to non-target birds from continuous exposure to higher levels of Cry1F protein.

RECOMMENDATIONS: A six week study with 60 to 70% corn in the diet is necessary to assess hazards from chronic exposure of wild and domesticated fowl.

2) Mammalian Wildlife

Since the anticipated exposure of mammalian wildlife is considered high, risk to wild mammals from Bt Cry1F is a potential concern. Direct wild mammal testing, however, is required only when human toxicology data are inadequate for assessment of hazard to wild mammals. The human health effects data submitted to EPA indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. In light of this toxicology information, no risk to mammalian wildlife is expected.

3) Plants

Since the active ingredient in this product is an insect toxin (*Bt* endotoxin) that has never shown any toxicity to plants, the plant toxicity studies have been waived.

4) Nontarget Beneficial Organism Studies

a) Honey Bees

The reviewed capped honey bee brood cell study where larvae were fed Cry 1F corn pollen and pure Cry1F protein showed normal larval development and emergence of healthy adult honey bees. This study shows that at levels higher than the expected environmental exposure, the proposed use of Cry1F protein in corn is not likely to have any measurable deleterious effects on the honey bee (*Apis mellifera)*. The data showed no significant difference between treatment mortality or behavior

change between the dosed and control replicates. As a result, no discernible detrimental effects to honey bees are expected from the proposed uses of the Cry1F producing corn. The data adequately address potential toxicity concerns for foraging honey bees exposed to Cry1F protein expressed in corn pollen in the field. In addition, since corn is wind pollinated, few honey bees are expected to be exposed.

b) Lady beetle predator:

Adult lady beetles (*Hippodamia convergens)* fed a concentration of Bt Cry1F protein at 15x the expected rate found in corn pollen resulted in no mortality or signs of toxicity over a 29 day period. Therefore, the NOEC was determined to be >15x the concentration of Cry1F found in pollen and the LC_{50} was determined to be > 480 ppm a.i (the test concentration). The submitted study shows that corn containing the Cry1F protein should not cause significant adverse effects to lady bird beetle predators. The test insects were exposed to a dose of active ingredient approximating the amount that would be ingested by the beetles feeding on aphids under field conditions. As a result, no discernible beneficial beetle population effects are expected from the proposed uses of the Cry1F producing corn. This conclusion is confirmed by adult and larval lady beetle abundance found in the field census study. These studies adequately address potential concerns for Cry1F protein expressed in corn to beneficial beetles.

c) Green lacewing

Green lacewing larvae fed a concentration of Bt Cry1F protein at 15x the expected rate found in corn pollen resulted in no mortality or signs of toxicity due to feeding on Cry1F over a 13 day period. Therefore, the NOEC was determined to be >15x the concentration of Cry1F found in pollen and the LC_{50} was determined to be > 480 ppm a.i (the test concentration). These laboratory findings do not show significant detrimental effects and provide data that show a lack of risk to beneficial insects at Cry1F levels that will be encountered in the field use situation. These findings confirm published field studies on the effects of *B.t.* crops on insect predators showing no significant differences in the density of beneficial insects, including green lacewings. The conclusions are also confirmed by the adult and larval green lacewing abundance found in a field census study submitted with this application.

d) Parasitic wasp

Parasitic Hymenoptera *(Brachymeria intermedia*) fed a concentration of Bt Cry1F protein at 10x the expected rate found in corn pollen showed no mortality or signs of toxicity over a 12 day period. Therefore, the NOEC was determined to be >10x the concentration of Cry1F found in pollen. The LC_{50} was determined to be > 320 ppm a.i (the test concentration). As a result, no adverse effect to parasitic wasps are expected from field exposure to Cry1F protein producing corn. The conclusions are also confirmed by the parasitic wasp abundance found in a field census study submitted with this application

e) Monarch butterfly

An additional scientifically sound study submitted by Dow AgroSciences showed that Cry1F is non-toxic to neonate monarch butterfly larvae when fed a 10,000 ng/mL diet dose. First instar larval weight and mortality were recorded after seven days of feeding. There was no mortality to monarchs fed 10,000 ng/mL diet, the highest rate tested. There was some growth inhibition at 10,000 ng/mL diet. Since pollen doses equivalent to 10,000 ng/mL diet are not likely to occur on milkweed leaves in nature, it can be concluded that Cry1F protein will not pose a risk to monarchs.

RECOMMENDATIONS: The conclusions should be confirmed by providing data showing that the amounts of Cry protein found in pollen on milkweed leaves in the field are at concentrations less than the 10,000 ng/mL diet used in this study. The NOEC of pollen on milkweed leaves also has to be determined.

f) Non-target Insects in the Field

A field study was conducted to determine whether Cry1F Bt corn had any significant negative impact on natural non-target insect populations. Results from a field evaluation study indicate that the transgenic corn lines 1507 and 1360 do not adversely affect the number of beneficial arthropods in the field. In general line 1507 showed larger numbers of beneficial insects. Beneficial insects counted in this study were: lady beetles (*Cycloneda munda* & *Coleomegilla maculata*), predacious Carabids, brown lacewings (Hemerobiidae), green lacewings (*Chrysoperla plorabunda*), minute pirate bugs (*Orius insidiosus*), assassin bugs (Reduviidae), damsel bugs (Nabidae), Ichneumonid and Braconids (parasitic wasps), damselflies and dragonflies, and spiders. Data included counts of adult and larval lady beetles and lacewings. This field census study adequately addresses potential concerns for Cry1F protein expressed in corn to non-target insect populations.

RECOMMENDATIONS: It is recommended that the beneficial insect monitoring should continue into the first few years of commercial use of Cry1F corn crops to confirm the single season "no effects" findings and to gather data on long range non-target insect effects and abundance.

g) Earthworm:

The submitted data show that Cry1F protein has no measurable deleterious effects on earthworms, a representative beneficial soil invertebrate species. This suggests that the proposed uses of the Cry1F protein in corn are not likely to have any measurable population effects on beneficial soil invertebrates. The one limit test concentration of 2.26 mg Cry1F/kg dry soil represented more than 100X the estimated concentration present in the top six inches of an acre of soil following the incorporation of 25,000 senescent corn plants. This concentration is higher than any amount of Cry protein that may be present in the soil during any stage of the growing season (such as from root exudation). Based on the results of this study, Cry1F transgenic corn plantings will have no adverse effects on earthworms.

h) *Collembola*:

Since Collembola feed on decaying plant material in the soil, they may be exposed to Cry1F protein in corn found in the field. A study was conducted to determine if there may be adverse effects of Cry1F on Collembola. The study is scientifically sound and no treatment mortality or behavior change was observed between the dosed and control replicates after 28 days. The results of this study indicate that at levels that would reasonably be expected to be found in the field, collembola were not affected by chronic exposure to Cry1F protein. The exposure rates in this study are 1560-, 388-, and 79-fold-higher than the expected field concentration. The reviewed data show that *Bacillus thuringiensis* Cry1F corn protein has no measurable deleterious effects on collembola (*Folsomia candida*), a representative beneficial soil insect species. This indicates that the proposed uses of the Cry1F protein in corn are not likely to have any measurable population effects on beneficial soil insects.

c. Risk Characterization for Aquatic Animals

Aquatic species: There is no evidence for sensitivity of aquatic (including endangered) species to Cry proteins. Toxicity studies with *Daphnia magna*, a very sensitive aquatic test organism, show no hazard for fish or invertebrates exposed to either corn pollen or to bacterially expressed Cry1F protein. In addition, aquatic exposure from Bt crops is extremely small. A simple standard pond scenario (1-ha pond, 2-m deep draining a 10-ha watershed planted with corn) was used to develop a worst case EEC for Cry1F protein on the basis of corn pollen loadings from airborne pollen deposition and agricultural runoff. Airborne pollen deposition results in water concentrations of approximately 1.25 ng Cry1F/mL and the contribution of Cry1F to the pond through agricultural runoff is 0.15 ng/mL. Thus, total water concentration of 1.4 ng Cry1F protein/L is projected under worst case conditions

1) Aquatic Invertebrates

The major source of Bt Cry1F protein in fresh water would be corn pollen. Toxicity studies with corn pollen containing Cry1F proteins conducted using the sensitive aquatic indicator species *Daphnia magna* show the no-mortality concentration and NOEC to be >100 mg a.i./L. There were no overt signs of toxicity to daphnids exposed to 100 mg Bt Cry1F pollen/L. The amount of pollen tested was considered to well exceed field exposure. These data indicate that the expected environmental concentration of corn pollen from the proposed use of Cry1F protein in corn is not likely to have any measurable population effects on aquatic invertebrates.

2) Fish

The registrant has requested a waiver of freshwater fish testing for transgenic maize containing *Bacillus thuringiensis* var. *aizawai* (Bt) Cry 1F protein. The basis of the waiver is the lack of significant exposure to fish and the low content of Cry1F protein in corn kernels in commercially manufactured fish diets (in aquafarms). Submitted data show that following processing there were

undetectable levels of Cry1F protein in fish food containing Cry 1F maize. The submitted data are sufficient to conclude that the low aquatic EEC and the lack of measurable concentrations of Cry1F protein in commercial fish diets are unlikely to present hazardous exposures to fish. Accordingly the registrant's request to waive fish toxicity studies is
acceptable.

3) Estuarine and Marine Animals

The Estuarine fish study was not required for this product because of very low or no potential for exposure.

d. Impacts on Endangered Species

The primary route of exposure to Cry1F protein in corn is through ingestion of corn tissue. There are no reports of threatened or endangered species feeding on corn plants, therefore such species would not be exposed to corn tissue containing the Cry1F protein. Since Cry1F corn pollen have shown no toxicity at the expected environmental concentration rates (EEC) to mammals, birds, plants, aquatic species, insect and other invertebrate species tested a "may effect" situation for endangered land and aquatic species is not anticipated given the current use pattern for this product. In its evaluation of endangered and threatened species, EPA considered all of the species listed in the Greenpeace and Environment Defense Fund petitions. In addition, EPA does not expect that any threatened or endangered plant species will be affected by outcrossing to wild relatives or by competition with such entities. Hybrid corn does not exist in the wild, nor are there wild plants that can interbreed with corn in the United States.

Because of the selectivity of Cry1F protein for lepidopteran species, endangered species concerns are mainly restricted to the order Lepidoptera. The majority of endangered lepidopteran species have very restricted habitat range that does not encroach on corn production areas. For example, Mitchell's satyr butterfly occur in wetlands fed by seeps and springs known as fens, and their larvae, which are present throughout the summer, feed primarily on sedges. No Mitchell satyr populations have been seen in close proximity to corn fields.

Examination of an overlay map showing the county level distribution of endangered lepidopteran species (as listed by the U.S. Fish and Wildlife Service) relative to corn production counties in the US, shows that they do not occur in agricultural areas where corn is grown, nor is corn considered a host plant for these species. The overlay map when combined with restricted habitat range clearly indicates that any potential concern for endangered or threatened butterfly species, including those listed in the Greenpeace petition is restricted to the Karner blue butterfly.
The Karner blue is found along the northern extent of the range of wild lupine, where there are prolonged periods of winter snowpack, primarily in parts of Wisconsin, Michigan, Minnesota, Indiana, New Hampshire and New York. The Karner blue requires wild lupine *(Lupinus perennis)* as an oviposition substrate and larval food source, while the adults feed on wild flowers. Wild lupine does not occur in corn fields, although there are anecdotal reports of wild lupine growing 'within a couple of hundred meters of corn fields. Wild lupine grows on dry, sandy soils in pine

barrens, oak savannah, forest trails and previously disturbed habitats such as utility rights-of-way, military installations, airports, highway corridors, sand roads and abandoned sand pits. There are recent reports that wild lupine may, in rare instances, grow in the vicinity of corn fields, especially in cases where the field may have been fallow in the previous season. However, there are no reports of Karner blue larvae or wild lupine within one meter of corn fields.

Karner blue oviposition overlap with corn pollen shed is also minimal. Although first generation Karner blues emerge in mid-April, prior to pollen anthesis, second generation larvae emerge in June-July when there may be some overlap with pollen-shed. However, there should be no risk of Karner blue exposure to maize pollen because larvae typically occur on wild lupines in full sunlight in open areas of savannas or barrens and not within corn fields.

Because Cry1F protein is active against Lepidoptera, some activity against the Karner blue at high dose levels would not be surprising. However, data on the levels of Cry1F pollen exceeding the NOEL inside the 1 meter corn field perimeter are not available. Testing of Karner blue larvae directly is difficult due to its endangered status. Although close relatives of the Karner blue butterfly are available, data from related lepidopteran species do not predict susceptibility to low levels of Bt proteins, even within the same genus. Since susceptibility of the Karner blue is not necessarily equivalent to other species from the genus Lycaeides and tests cannot be conducted with the Karner blue, determining a NOEL is difficult to impossible. However, the Karner blue is probably no more sensitive to Cry1F than monarch butterflies and will not consume toxic levels of Bt in the field.

Conclusion: Exposure of Karner blue butterflies to harmful levels of Cry1F corn pollen is not expected. Likewise, a review of the preferred habitats of other lepidopteran species listed as endangered by the U.S. Fish and Wildlife Service, including the endangered Mitchell satyr butterfly, indicates that no exposure to harmful levels of Cry1F protein containing pollen will take place. Therefore, EPA believes that this action will have no effect on listed species. However, because of the lack of direct testing of Cry1F effects on the endangered Karner blue butterfly *(Lyceides melissa samuelis)* and recent information on the possibility of exposure of the Karner blue to corn pollen under certain rare circumstances (such as replanting of fallow fields), at this time geographic restrictions are needed for this product to eliminate potential exposure of Karner blue butterflies to Cry1F corn pollen. Without geographic restrictions, at this time it is not possible to make a definitive "no effect" finding without a consultation with the US Fish and Wildlife Service. The Agency plans to conduct further work to understand the extent to which the practice of replanting fallow fields might expose Karner blue butterflies to Bt corn pollen.

4. Endangered Species Statement

Of particular concern is the endangered Karner blue butterfly (*Lycaeides melissa samuelis*) with populations in Illinois, Indiana, Michigan, Minnesota, New Hampshire, New York, and Wisconsin. Because of the potential for *B. t.* Cry protein containing pollen to affect Lepidoptera adversely, Cry1F maize must not be near habitats of the Karner blue butterfly in the following counties where the Karner blue butterfly is known to exist in scattered populations: Illinois - Lake; Indiana - Porter and Lake; Michigan - Allegan, Lake, Monroe, Montcalm, Muskegon, Newaygo and Oceana;

Minnesota - Anoka and Winona; New Hampshire - Merrimack; New York - Albany, Saratoga, Schenectady and Warren; Wisconsin - Adams, Barron, Burnett, Chippewa, Clark, Dunn, Eau Claire, Green Lake, Jackson, Juneau, Kenosha, Marquette, Menominee, Monroe, Oconto, Outagamie, Polk, Portage, Sauk, Shawano, St. Croix, Waupaca, Waushara, Wood; (this list is from the Wisconsin Statewide Karner Blue Butterfly Habitat Conservation Plan and Environmental Impact Statement). Although it is unlikely that sufficient Cry1F expressing pollen would accumulate on the wild lupine (*Lupinus perennis*) that constitutes the sole food source for the butterfly larvae, this precaution is needed in the lack of adequate data from the field indicating the precise proximity of wild lupine to corn fields in the above named counties.

D. INSECT RESISTANCE MANAGEMENT

This assessment is based upon the current understanding of the science. The Agency has received the October 18-20 FIFRA Scientific Advisory Panel (SAP) report and is doing a complete reassessment of the risks and benefits of Cry1Ab- expressing corn. This reassessment will address insect resistance management (IRM) needs based upon current information. Because of the potential cross-resistance between Cry1F and Cry1Ab expressing field corn, Cry1F will be subject to IRM requirements addressed in the Bt corn reassessment to be completed in 2001. The BPPD IRM team agrees with Dow AgroSciences and Pioneer Hi-Bred that all seed manufacturers and extension specialists should convey the same uniform IRM message to growers for all Bt field corn products in which there is possible cross-resistance. Therefore, Cry1F IRM requirements should coincide with the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) requirements and message.

Pest Biology:

Pests susceptible to Cry1F include the European corn borer (ECB, *Ostrinia nubilalis*), southwestern corn borer (SWCB, *Diatraea grandiosella*), black cutworm (BCW, *Agrotis ipsilon*), fall armyworm (FAW, *Spodoptera frugiperda*), lesser cornstalk borer (LCSB, *Elasmopalpus lignosellus*), sugarcane borer (SCB, *Diatraea saccharalis*), and to a lesser extent the corn earworm (CEW, *Helicoverpa zea*). A high dose has been demonstrated for ECB and a high level of efficacy was found for SWCB, FAW, and BCW. Unlike currently registered Cry1Ab field corn hybrids, Cry1F is highly efficacious against the BCW and FAW. Otherwise, the relative susceptibilities of the insects tested to Cry1F were LCSB>SWCB>SCB. Although the LCSB, SCB, and CEW were shown to be susceptible to Cry1F, these pests are not listed on the label. The CEW is susceptible to Cry1F at approximately the same rate it is susceptible to Cry1Ab. However, since CEW is susceptible to Cry1F, IRM requirements and research needs should be addressed. Current Bt corn registrations require annual submission of research data by January 31. Cry1F is subject to the same research requirements that are outlined in the table below. However, these requirements may change upon completion of EPA's comprehensive reassessment of B.t. plant-incorporated protectants. In addition, an LC_{50} should be determined for SWCB, CEW, FAW, and BCW if a colony can be established.

Summary of Data Needed to Improve Insect Resistance Management Strategies for Bt Corn Products

Data	Pests
Pest Biology: e.g., larval movement, adult movement, mating behavior, pre- and post-mating dispersal, ovipositional behavior, fitness, and overwintering habitat and survival	ECB, SWCB and CEW
North to South Movement	CEW
High Dose Verification (using 1998 SAP techniques)	ECB and SWCB
Resistance Allele Frequency	ECB, SWCB and CEW
Cross-Resistance - Cry1F, Cry2A, Cry1A proteins	ECB, SWCB and CEW
Evaluation (field studies and models) of Refuge Options (20% external refuge (sprayable) v. 20% in-field) - [Issues to consider: production of susceptible insects (500:1 ratio) in insecticide treated and non-insecticide treated refuges, adequacy of size, structure, and deployment of the refuge, rotation of refuge.]	ECB, SWCB and CEW
Collection of Baseline Susceptibility Data and Validation of Discriminating/Diagnostic Dose	ECB, SWCB and CEW
Evaluation of Resistance Monitoring Techniques, e.g., discriminating v. diagnostic dose, F_2 screen, sentinel plots, gene mapping	ECB, SWCB and CEW
Grower Compliance - more detailed information on refuge (%, deployment, and management)	ECB, SWCB and CEW

Significant pest biology research has been conducted for ECB as it relates to IRM. Work with larval movement, adult movement, mating behavior, ovipositional behavior, and host range have established critical parameters for ECB refuge, although more research is needed in these areas. A less extensive pest biology knowledge base is available for CEW and SWCB. As is the case with ECB, additional IRM-related research is needed for SWCB, CEW, BCW, FAW and other secondary corn pests to increase confidence that the IRM plans will be effective at reducing the likelihood that insects will become resistant to Bt corn.

<u>High Dose</u>:

The February, 1998 FIFRA Scientific Advisory Panel (SAP) Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management determined that a high dose/refuge strategy is necessary to mitigate resistance of stalk boring Lepidoptera in Bt corn (meeting held on February 9-10, 1998. Docket # OPPTS-00231). The SAP determined that a high dose (defined as 25× the dose necessary to kill all susceptible insects) should be verified by two of five techniques outlined in the final report (http://www.epa.gov/scipoly/sap/1998/february/finalfeb.pdf).

Ideally, high dose should be evaluated for all susceptible pests, so that appropriate resistance management strategies can be developed. At a minimum, high dose should be determined for the major target pests of Cry1F corn (ECB and SWCB). The registrants claim that a high dose for Cry1F event TC1507 was determined by the fourth and fifth methods described by the SAP. Method four is similar to method 3, but would use controlled infestation with a laboratory strain of the pest that had an LD50 value similar to field strains. Method 3 involves surveying large numbers of commercial plants on sentinel plots in the field (e.g., sentinel sweet corn method) to make sure that the cultivar is at the $LD_{99.99}$ or higher to assure that 95% of heterozygotes would probably be killed. With this approach *Bt* sweet corn hybrids are used to attract high densities of ECB and cotton bollworm (*Helicoverpa zea*)(Boddie)) (CBW/CEW) moths, sampling can be limited to sweet corn ears in the *Bt* plot (ca. 1/4-1/2 acre block), and a frequency of resistant phenotypes can be estimated as the ratio of density of larvae/plant in *Bt* sweet corn to density of larvae/plant in an adjacent planting of non-*Bt* sweet corn (Andow and Hutchison, 1998; Hutchison, unpublished data). With the fifth method identified by the SAP, it should be determined if an older instar of the targeted pest could be found with an LD50 that was about 25-fold higher than that of the neonate larvae. If so, that stage could be tested on the crop plants to determine if 95% or more of the older stage larvae were killed.

Although the fourth method was used, Bt field corn rather than sweet corn was planted because Cry1F sweet corn does not exist. The SAP recommended sweet corn be used since it is more attractive to ECB than field corn. It was also confusing how many and why neonates from other trials were used in the method four verification. Overall, a high dose for ECB has been demonstrated in this report for event TC1507. Additional high dose data are needed for the SWCB.

Refuge:
A refuge should be designed to produce 500 susceptible insects for every one potentially resistant insect. This Cry1F registration will be subject to the same requirements as the Agricultural Biotechnology Stewardship Committee (ABSTC) and October 2000 SAP report that will consider refuge needs in an IRM strategy. Refuges planted to mitigate resistance to Cry1F field corn should be identical to those needed for Cry1Ab corn and should consist of agronomically similar non-Bt field corn varieties. The Bt and non-Bt field corn varieties should be cared for and managed in a similar fashion. The non-Bt refuges should be planted within the Bt field or in close proximity. In general, refuges may be planted as external blocks on the edges or headlands of fields or as strips within the Bt corn field. In-field strips should include multiple rows (at least 2-6) of non-Bt field corn and extend the full length of the field. Refuges should be treated as needed to control lepidopteran stalk-boring insects with non-Bt insecticides or other appropriate IPM practices. Insecticide use should be based on scouting using economic thresholds as part of an IPM program.

The issue of refuge proximity is a critical variable for resistance management. Refuges must be located so that the potential for random mating between susceptible moths (from the refuge) and possible resistant survivors (from the Bt field) is maximized. The USDA NC-205 North-Central regional research committee on ecology and management of European corn borer and other stalk-boring Lepidoptera has recommended to the Agency that all Bt corn should be placed within one half mile of the non-Bt corn refuge, but that refuge plantings within one quarter mile would be

even better (NC-205 letter to Dr. Janet Andersen, 5/24/99). However, the complete picture of ECB dispersal is still unknown and among females, mating is likely to occur (relatively) close to the site of pupal eclosion. In addition, information is needed on SWCB and CEW movement and mating behavior to fully understand optimal placement of refuges. This research should be conducted and submitted to the Agency as part of the annual research report.

Based upon NC-205 recommendations made from 1998-2000, and information regarding ECB resistance management acquired from models (Onstad & Gould 1998, Onstad & Guse 1999), a minimum of 20% non-Bt corn refuge is recommended for non-cotton growing regions (e.g., Corn Belt) that do not spray insecticides on a regular basis. A 20% non-Bt corn refuge in non-cotton growing regions was recommended by the ABSTC in April 1999 and mandated by EPA in 2000.

The October 2000 SAP Subpanel is currently drafting a report that considers whether a larger refuge, e.g., 40% non-Bt corn refuge, would decrease the risk of insect resistance in non-cotton growing regions that spray insecticides on a regular basis (e.g., High Plains for SWCB). This area needs to be clearly identified on a county basis or triggers developed to indicate when higher insecticide use poses a greater risk to resistance. A larger refuge may be needed in this area because of the increased risk of resistance created when insecticides are used, thus killing insects susceptible to Bt. Currently, EPA has mandated a 20% non-Bt corn refuge in all non-cotton growing regions.

Cry1F corn grown in cotton-growing regions, including the northern cotton growing region, should plant a minimum 50% non-Bt corn refuge that may be treated only as necessary with non-Bt insecticides. This larger refuge size in cotton-growing regions is needed due to increased concerns regarding the development of CEW resistance in cotton growing regions especially those regions growing Bt cotton.

Monitoring:
The monitoring strategy submitted for this registration is not adequate because it only addresses grower monitoring for unexpected damage. A resistance monitoring strategy for Bt corn is needed to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. Techniques such as the F_2 screen, and sentinel plots need to be thoroughly investigated for their feasibility as resistance monitoring tools in addition to the currently used discriminating dose concentration assays. The ABSTC must submit research to the Agency regarding the feasibility of conducting the F_2 screen by March 2001. In addition, baseline susceptibility to Cry1F data should be developed for ECB, SWCB, and CEW.

The ABSTC proposed a monitoring strategy in March 2000 for ECB, CEW, and SWCB in Cry1Ab field corn. The ABSTC plan to monitor for ECB, SWCB, and CEW resistance should also be used for Cry1F field corn in addition to the grower monitoring presented in the Dow AgroSciences/Pioneer Hi-Bred submission. The ABSTC plan focuses resistance monitoring in areas where Bt corn market penetration is highest as well as areas with the highest insecticide use. The

ABSTC plan includes the identification of counties growing more than 50,000 acres of field corn (Bt and non-Bt) to focus monitoring efforts. ABSTC's proposed plan is designed to detect resistance when it reaches 1 - 5% (a level that allow for detection of resistance before field failures occur). Four corn-growing regions were identified and monitoring for each pest will occur in the regions in which the pests are prevalent. When possible, at least 200 first or second flight adults (100 females), 100 second flight egg masses, and 100 diapausing larvae per site will be collected in each region, though insect population levels may limit the number collected. The ABSTC sampling strategy should be adequate, although the program would be improved if sampling locations can be separated by a sufficient distance to reflect discreet pest populations.

The BCW and FAW are polyphagous insects that are considered secondary pests of field corn. BCW feed on a wide range of cultivated crops including turfgrass, field and vegetable crops. FAW feed on over 80 host plants including corn and vegetables, but they prefer grasses. Both of these pests overwinter in the south and migrate north. FAW only overwinters in south Texas and south Florida. Due to their polyphagous nature and migratory patterns, there is a great amount of gene flow. There have not been reports of resistance to insecticides by either of these pests in the U.S. For these reasons, there is not a concern of FAW or BCW becoming resistant to Bt field corn. In addition, it would be difficult to survey field corn for unexpected damage since these pests will probably cause some damage prior to being killed by the Cry1F toxin (personal communication with Galen Dively 1/2001). Therefore, monitoring for BCW and FAW resistance is not necessary in field corn expressing the Cry1F protein. However, if there were to be large amounts of Cry1F field corn (>1000A) planted in the areas in which FAW overwinters (south Florida and south Texas), the Agency will reexamine whether there is significant selection pressure for FAW and BCW to develop Cry1F resistance.

Remedial Action:
Remedial Action was not adequately addressed by Dow AgroSciences and Pioneer Hi-Bred. Dow AgroSciences and Pioneer Hi-Bred proposed that all instances of resistance will be reported to the Agency and mitigation measures will be taken. Mitigation measures include ceasing sales in the affected counties, but Dow and Pioneer do not address continued monitoring in the area or the use of alternate control measures. The Cry1F remedial action plan should be identical to the ABSTC plan for Cry1Ab expressing field corn already approved by the Agency in 2000.

Remedial actions should include: informing customers and extension agents in the affected areas of suspected or confirmed resistance, increasing monitoring in the affected areas, implementing alternative means to reduce or control target pest populations in the affected areas, implementing a structured refuge in the affected areas, and cessation of sales in the affected and bordering counties until an effective local management plan approved by EPA has been implemented. During the voluntary suspension period, registrants may sell and distribute in these counties only after obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy will be coordinated by the Agency with other registrants and stakeholders.

Compliance:

Dow AgroSciences and Pioneer Hi-Bred do not believe 100% compliance is necessary as long as the large majority of growers comply with IRM requirements. Growers will need to sign a technology use agreement that outlines IRM requirements and acknowledges the grower's obligation to comply with them. The agreement will also state that growers received the Product Use Guide. This agreement may be a section of the growers order sheet or some other document or format. The grower agreement must be signed annually. Dow AgroSciences and Pioneer Hi-Bred recommend grower surveys to estimate the level of compliance, limiting non-compliant growers access to the technology, and implement additional education efforts will target non-compliant growers.

Cross-Resistance:
Cross-resistance, in which one toxin confers resistance to another, is an area of major concern for resistance management and poses risks to both transgenic Bt crops and microbial Bt insecticides. The most well-documented mechanism of cross-resistance with Bt occurs when two toxins share the same binding site (receptor) in the insect midgut (Tabashnik 1994).

Competitive binding experiments provided by the registrants showed that Cry1F may share a binding site in the ECB midgut with Cry1Ab or Cry1Ac, but not with the Cry9 proteins. This demonstrates that ECB has the potential for cross-resistance between Cry1F and Cry1Ab (currently registered Bt corn) and Cry1Ac (currently registered Bt cotton). Therefore, the IRM strategy mandated for Cry1Ab field corn should also be implemented for Cry1F corn. In addition, it is not recommended to stack Cry1Ab and Cry1F in the same corn hybrid.

Grower Education:
Dow AgroSciences and Pioneer Hi-Bred have adequately addressed grower education through training their trainers, Product Use Guides, field placards, slide presentations and publicity. The proposal they submitted to the Agency should be implemented. Growers are perhaps the most essential element for the implementation and success of any IRM plan as they will ultimately be responsible for ensuring that refuges are planted according to guidelines and that Bt fields are monitored for unexpected pest damage. Therefore, a program that educates growers as to the necessity of IRM and provides guidance as to how to deploy IRM should be an integral part of any resistance management strategy. Ideally, the educational messages presented to growers should be consistent (among different registrants) and reflect the most current resistance management guidelines for current Bt corn registrations. Specific examples of education tools for growers can include grower guides, technical bulletins, sales materials, training sessions, Internet sites, toll-free numbers for questions or further information, and educational publications.

Annual Reports:

Written reports on various aspects of IRM, submitted on an annual basis to EPA, are of great aid in the evaluation of the success of resistance management for Bt corn. Reports should be submitted to the Agency on an annual basis on Bt corn sales/market penetration, IRM-related research, and grower education. In addition to these reports, it would be particularly useful to receive reports from Bt corn registrants on grower compliance and resistance monitoring.

References:

Onstad, D.W. and F. Gould. 1998. Modeling the dynamics of adaptation to transgenic maize by European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 91(3): 585-593.

Onstad, D.W. and C.A. Guise. 1999. Economic analysis of transgenic maize and nontransgenic refuge managing European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 92(6): 1256-1265.

E. BENEFITS

Criteria for approval

The criteria for a determination as to whether registration of a pesticide chemical is in the public interest are set forth in a Federal Register notice dated 3-5-1986 volume 51, No.43 (OPP-32500; FRL-2977-2) Conditional Registration of New Pesticides. Thus, there is a presumption that registration of a pesticide chemical is in the public interest if one of the following criteria is met: (i) the use is for a minor crop; (ii) the use is a replacement for another pesticide that is of continuing concern to the Agency; (iii) the use is one for which an emergency exemption under FIFRA Section 18 has been granted for lack of an alternative pest control method, or (iiii) the use is against a pest of public health significance. Notwithstanding whether a registration of a pesticide chemical may be presumed to be in the public interest, EPA may determine that such a registration is in the public interest on the basis of the following criteria: (i) there is a need for the new chemical that is not being met by currently registered pesticides; (ii) the new pesticide is comparatively less risky to health or the environment than currently registered pesticides; or (iii) the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non chemical techniques.

Summary of Finding

Registration of *Bacillus thuringiensis* subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in corn is in the public interest because the new pesticide is comparatively less risky to health or the environment than currently registered pesticides and the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques.

The registered alternatives commonly used to treat the target pest complex protected by Cry1F are restricted use for the most part. They have precautionary label statements such as extremely toxic to fish and aquatic organisms, wildlife and require protective clothing for workers. The specific organophosphate and pyrethroid pesticides likely to be replaced are ranked in the top 15 of all pesticides with respect to reported incidents of mortality to non-target wildlife. Many of these

products also control corn root worm, which is the most significant pest of corn and is frequently treated along with the target pest complex of Cry1F. Compared to other Bt corn products, growers are likely to choose Cry1F protected corn due to better product performance and broader spectrum of control. Cry1F protected corn is also expected to be economical on some unprotected fields and provide insurance against the risk of crop loss and the need to replant. But without root worm protection, the use of Cry1F to reduce conventional pesticide use is limited.

At product maturity, grower benefits of Cry1F protected corn are estimated to be between $28 to $81 million per year on 7.3 to 12.5 million acres of field corn. The range depends upon the technology fee, from $7.50 to $13.13/acre. Grower benefits are not a prediction since it does not include the effects of other technological innovations or competitor reactions on the pricing of pest control products. It does not include the effects of stacked genes offering multiple benefits, new competitive products, or the effects of increased competition in the corn insecticide market. Increased competition should offer growers more choice and lower the cost of pest control. The benefits are the incremental improvement to grower profits compared to current practice. All costs are eventually passed along to consumers in the long run, but this review did not deal with the complex topic of the dynamics of when that will occur.

APHIS Environmental Assessment

EPA agrees with the following conclusions from the environmental assessment conducted by APHIS.

"The EA addressed the potential for impacts to the human environment that might be incurred from an APHIS determination....."

"APHIS believes that cultivation of Bt Cry1F corn line has the potential to further reduce insecticide applications targeted not only for the European corn borer and other corn borers, but for cutworm and armyworms as well, provided these insecticides are not also being applied to control the corn root worm. Because many of these insecticides are more toxic to humans and non target organisms...a reduction in their use should provide benefits to the environment as well as to humans, particularly farm workers and their children who are at a higher risk from exposure"

Benefit claims made in PIF documents submitted for review

The registrants believe that Cry1F-protected corn is clearly in the public interest and provide data to support the following claims:

Cry1F provides highly efficacious control of key Lepidopteran pests of field corn

Cry1F provides a broader spectrum of pest control than other Bt corn products

Cry1F hybrids provide comparable or superior pest control compared to existing Bt corn products for all pests.

The use of Cry1F is expected to reduce the use of more toxic chemical insecticides

Cry1F will reduce level of mycotoxin in corn.

Cry1F protein presents a very low risk to monarch butterflies (superior to Event 176)

Cry1F corn does not contain an antibiotic resistance gene and thus meets the long term criteria established for use in Europe

Cry1F hybrids are predicted to gain significant market share within the first five years after registration as a result of benefits to growers

The registrants have submitted data to support the economic benefits to growers. This included efficacy trials comparing Cry1F to non Bt hybrids, yield and other agronomic characteristics of Cry1F hybrids against their respective non transgenic counterparts, and economic models to compare the benefits of various insect control strategies under different insect pressures. Data to support claims for mycotoxin reduction were not submitted.

The registrant submitted data indicate that Cry1F protected corn offers excellent control of European corn borer (ECB), southwestern corn borer (SWCB), fall armyworm (FAW), black cutrworm (BCW), and suppression for the corn earworm(CEW).

Changing current pest management practices

Growers may adopt Cry1F protected corn in three situations:

1) Replace current Bt products
2) Replace chemical insecticides
3) Protect against the risk of replanting due to loss from unprotected corn

The most popular corn insecticides currently used (1998/99 data) to treat the pest complex controlled by Cry1F protected corn are identified in the Table 1 below. Permethrin and terbufos have been identified in the top 15 pesticides reported in incidents of mortality to non target aquatic organisms (EPA ecological incident monitoring system). Chlorpyrifos has been found in surface water monitoring data, detected in sediment or biota at more than 10 percent of total sites. (USGS, National Water Quality Assessment Program, NAWQA). With the exception of Chlorpyrifos, all insecticide alternatives are restricted use, extremely toxic to wildlife, and require protective clothing for workers, as shown in Table 1.

Table 1. Effects of Insecticide Alternatives to Cry1F

Common Name	Precautionary Label Language
PERMETHRIN	Restricted use, extremely toxic to fish and aquatic organisms, highly toxic to bees, protective clothing for workers
CYHALOTHRIN-LAMBDA	Restricted use, extremely toxic to fish and aquatic organisms, highly toxic to bees, protective clothing for workers
CHLORPYRIFOS	Toxic to birds and wildlife and extremely toxic to fish and aquatic organisms
TEFLUTHRIN	Restricted use, very highly toxic to freshwater and estuarine fish and invertebrates. May pose a hazard to endangered species.
TERBUFOS	Restricted use, extremely toxic to fish and wildlife, protective clothing

Source: Registrant submissions and Crop Data Management Systems
http://www.cdms.net/manuf/AgLinks.asp

About 24 million acres of corn are treated with an insecticide, which is 30% of the 80 million planted acres.

The target pest infestation occurs in the Southeast for fall armyworm, Midwest for black cutworm, and Upper Midwest for the European corn borer. Potential for use reduction occurs when growers can substitute Cry1F protected corn for the conventional pesticides (shown in Table 2). The reference to low, medium and high in the potential for use reduction column refers to the chemical treatments of the target pest compared to the total chemical treatments. Low is for less than 15%, medium between 15% and 30%, and high is above 30%.

Table 2. Potential for insecticide use reduction on corn

State	Planted (000's)	Pct treated (000's)`	Lbs ai (000's)	Potential for use reduction
Illinois	10,800	38	1,833	medium
Iowa	12,100	25	2,462	medium
Missouri	2,650	38	218	high
Nebraska	8,600	39	1,295	low
Kentucky	1,320	50	22	high
Indiana	5,800	36	1,156	low
Ohio	3,450	7	98	medium
Minnesota	7,100	11	280	medium
Texas	1,950	54	458	low
Kansas	3,150	32	385	low
Wisconsin	3,600	31	473	low
Colorado	1,230	45	479	low
South Dakota	3,600	18	520	medium
Michigan	2,200	22	214	medium
North Carolina	750	35	222	low
Total for states surveyed	68,300	30	10,115	

Source: Agricultural Chemical Usage 1999 Field Crops Summary, NASS, and EPA estimates.

Estimating grower economic benefits

Registrant submitted data are used to estimate grower economic benefits. A Model grower economic analysis is provided for different typical grower pest management situations:

High risk for Black cutworm, moderate risk for European corn borer.
Moderate risk of Black cutworm and southwestern corn borer.
High risk for fall armyworm.

Information is provided on TC1507 to represent Cry1F. Alternative options include no treatment, preventative and rescue insecticide treatments, replant seed, and other Bt corn hybrids Event 176 (Mycogen) and CBH 351(Aventis).

Table 3. Model Grower Economic Analysis: Returns over variable cost

Scenario	Best alternative approaches	Maximum advantage/acre
High risk for Black cutworm, moderate risk for European corn borer	CBH-351, Rescue	$23.36
Moderate risk of Black cutworm and southwestern corn borer	CBH-351, Rescue	$18.06
High risk for fall armyworm.	Event 176, CBH-351	$48.46

The Monte Carlo simulation model described in the Bt reassessment is used to estimate adoption rate and grower benefits for Cry1F protected corn (see Bt reassessment documents for a description of the methodology). The advantage of Cry1F protected corn over the next best alternative is set at $25 per acre, a sort of 95% upper limit. This is closer to the cutworm and corn borer situations than the fall armyworm since these cases represent the bulk of chemical treatments. The technology fee is stated to be between $7.5 to $13.13/acre. Bt related costs is assumed to be $10/acre, an estimate also used in Bt reassessment. These costs cover refuge requirements and marketability concerns and apply to situations where Cry1F replaces chemical control or no control.

Acres at risk is estimated to be 25 million acres, based on the states affected and the extent of area infested. Grower benefits could vary by an average of $3.90/acre to $6.51/acre. The very wide range is due to the wide range of the proposed technology fee, from $7.50 to $13.13 per acre.

It should be noted that these annual benefits would occur at product maturity, or 3 to 5 years after commercialization. The analysis does not consider possible stacked products which offer multiple protections and efficiencies, the effect of new competitor products, or the impact of increased competition on overall market equilibrium conditions.

Table 4. Summary of Estimated Grower Benefits for Cry1F

Technology fee	Grower Benefits per acre
$13.13/acre	$3.90
$7.50/acre	$6.51

Based on: 1) 25 million acres at risk for ECB, BCW, SWCB, and FAW; 2) Current pricing for competitive pest control products; and 3) field corn market price/bushel of $2.25. Note that corn prices are volatile. Lower prices reduces the economic value of pest protection and would lower the acreage of Cry1F.

III. Data Gaps and IRM Requirements

A. Data Gaps

The following data was determined necessary to complete the pending products' database for registration until September 30, 2001.

1) A longer soil degradation study in actual field soil.

There is no evidence to indicate that prolonged exposure to trace amounts of Cry protein in the soil affects non-target organisms. The submitted data do not, however, sufficiently address the issue of residual Cry protein accumulation in the soil. The soil degradation study should be carried out for a longer period of time to determine the duration and the amount of residual Cry 1F protein in agricultural soil. Also, the soil used in the study should be actual field soil containing the microbial flora normally found in the field. This will give a more accurate rate of degradation of the Cry protein in the agricultural environment because microbial populations in the rhizosphere are commonly 100 fold higher than in bulk soil. Bulk soil generally does not support populations of microorganisms as high as those in the rhizosphere or those in soils with high organic content (plant residues). In addition, field soil high in organic content should result in lower (if any) soil binding of Cry proteins.

2) Confirmatory Monarch butterfly data.

An additional scientifically sound study submitted by Dow AgroSciences showed that Cry1F is non-toxic to neonate monarch butterfly larvae when fed a 10,000 ng/mL diet dose. First instar larval weight and mortality were recorded after seven days of feeding. There was no mortality to monarchs fed 10,000 ng/mL diet, the highest rate tested. There was some growth inhibition at 10,000 ng/mL diet. Since pollen doses equivalent to 10,000 ng/mL diet are not likely to occur on milkweed leaves in nature, it can be concluded that Cry1F protein will not pose a risk to monarchs.

The conclusions should be confirmed by providing data showing that the amounts of Cry protein found in pollen on milkweed leaves in the field are at concentrations less than the 10,000 ng/mL diet used in this study. The NOEC of pollen on milkweed leaves also has to be determined.

3) Continuation of beneficial insect field monitoring.

The beneficial insect monitoring should continue into the first few years of commercial use of Cry1F corn crops to confirm the single season "no effects" findings and to gather data on long range non-target insect effects and abundance.

4) Insect resistance management data.

The registrants will confer with the EPA as the registrants develop various aspects of its resistance management research program. The registrants agree, as a condition of these registrations, to generate data and to submit annually progress reports on or before January 31st each year on the following areas as a basis for developing a long-term resistance management strategy which include:

a) The registrants must submit available research data on CEW relative to resistance development and the registrants' plans for producing resistance predictive models to cover regional management zones in the cotton belt based on Helicoverpa zea biology and cotton, corn, soybeans, and other host plants. These models must be field tested and must be modified based on the field testing performed during the period of the conditional registration. EPA might modify the terms of the conditional registration based upon the field testing validation of the model and might require refuge in the future. EPA notes that there is some scientific work and even some models for H. zea on other crops in at least NC and TX that could be used for reference. EPA wants to be in close communication with the registrants as the model development and testing is ongoing. The requirement for development of resistance predictive models may be modified if the registrants provide the results of research that demonstrates resistance to CEW would have no significant impact on the efficacy of foliar Bt products and other Bt crops. Actual usage data of Bta on crops to control specific pests as well as successes and failures and field validated research would be necessary to support such a waiver request.

b) ECB pest biology and behavior including adult movement and mating patterns, larval movement, survival on silks, kernels, and stalks, and overwintering survival and fecundity on non-corn hosts. A combination of a comprehensive literature review and research can fulfill this condition.

c) The feasibility of "structured" refuge options for ECB including both "block" refuge, "50-50 early/late season patchwork;" research needs to be done in both northern and southern areas on ECB as well as CEW.

d) Development of a discriminating concentration (diagnostic concentration) assay for field resistance (field screening) for ECB, CEW and SWCB. Sampling will be done in accordance with the Industry Plan to determine if increases in Cry1F toxin tolerance are occurring before crop failures develop. Increased tolerance levels need to be identified before field failure occurs. In monitoring for tunneling damage, the number of trivial tunnels may be less indicative of resistance development than the total extent of tunneling damage (e.g. length of tunnels). The extent of tunneling damage must be monitored as well as the number of tunnels.

e) Effects of corn producing the Cry1F delta endotoxin on pests other than ECB, including but not limited to CEW, fall armyworm, and the stalk borer complex.

f) The biology of ECB resistance including receptor-mediated resistance and its potential effect on population fitness, as well as the effects on insect susceptibility to other Cry proteins.

g) You must assess the feasibility of using the F2 screen, sentinel plots, and in-field screening kits to increase the sensitivity of resistance monitoring in 2001. By January 31, 2002, you must provide the Agency with the results from these investigations.

h) You must implement a survey approach similar to the Iowa State University Bt Corn Survey (e.g., Pilcher and Rice, 1999) A statistically valid sample, as determined by Independent market research, of Bt corn growers in key states will be surveyed by a third-party. Bt corn growers will be included based upon a proportionately stratified random sample designed to balance the survey evenly across seed companies and geographies. In addition to demographic information, the survey will include questions related to insect resistance management such as:

1) What is your primary source of information on Bt corn?

2) What percentage of your acres were planted to Bt corn this year?

3) Are you following a recommended insect resistance management strategy?

4) If you plant most of your acreage to Bt corn, are you likely to scout your non-Bt corn for economically damaging populations of corn borers?

5) Did you treat your Bt corn acres with an insecticide?

6) What planting pattern did you use for your refuge?

- ° Planted Bt corn as one block in one field.
- ° Planted Bt corn in one block in every field.
- ° Split seed boxes in the planter and alternated every row or several rows with Bt and non-Bt corn in every field.
- ° Planted Bt corn in large strips alternated with large strips of a non-Bt corn hybrid.
- ° Planted Bt corn in an entire field and planted the border around the field with non-Bt corn.
- ° Planted pivot corners to non-Bt corn with the irrigated area of the field planted to Bt corn.

5) Analytical methods and method validation for the Cry1F protein in corn have been received and are acceptable, but additional confirmatory methods and standard post-registration EPA laboratory method validation are required.

Although, Cry1F protein plant root expression and exudation data and a 6 week avian feeding study with 60 -70% Cry1F corn in the diet were identified as deficiencies for a non-expiring full commercial use registration, they are not considered data gaps for a registration expiring on September 30, 2001. At this time in the reassessment process, these data have not been required of other Bt corn plant-incorporated protectant registrants.

B. IRM Requirements

The following requirements specified in the registrations for Cry1F event 1507 are based on the Agency's requirements for Cry1Ab expressing corn. This is due to the possibility of cross-resistance between Cry1Ab and Cry1F. Modifications of these requirements may result following the Agency's comprehensive reassessment of B.t. plant-incorporated protectants.

1) Several aspects of the Insect Resistance Management Plan will operate in synergy to promote grower compliance, however, the cornerstones of the compliance program must be the:

a) Grower Guides

Grower Guides and/or Product Use Guides must be submitted to the Agency at the time of distribution to growers. These Guides must be distributed to each seed customer and updated on an annual basis, as needed. The Guides provide complete information for growers regarding routine IRM practices that must be employed, and will be a primary educational and reference tool. Agreed-upon requirements and additional information that cannot be included in the Grower Guides for 2001 (e.g., because the requirements were enacted after printing and distribution of the Grower Guides) must be conveyed via supplemental communications to Cry1F field corn seed customers.

b) Stewardship Agreement (grower agreement).

Each grower who purchases Cry1F field corn seed must be required to sign a Stewardship Agreement, which will obligate the grower to follow the required IRM and non-target insect protection practices as specified in the Grower Guide/Product Use Guide and/or in supplements thereof.

c) A Strong and Multi-Pronged Grower Education Program.

A variety of methods must be employed to promote grower education and to continue to reinforce the need for adherence to all aspects of the IRM program.

d) Additional mechanisms must also be used to promote grower compliance, including:

Training of sales personnel, seed dealers and technical support staff. Coordination and reinforcement of IRM requirements through other organizations (e.g., NC-205, the Cooperative Extension Service,

USDA, National Corn Growers Assn. (NCGA), American Crop Protection Assn., Biotechnology Industry Organization, crop consultants and other crop professionals).

2) (Stewardship Agreements/Grower Agreements) will specify that growers must adhere to the refuge requirements as described in the Grower Guide/Product Use Guide and/or in supplements to the Grower Guide/Product Use Guide. Specifically, growers must plant a minimum structured refuge of at least 20% non-Bt corn. Insecticide treatments for control of European corn borer, corn earworm and/or Southwestern corn borer may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Instructions to growers will specify that microbial Bt insecticides must not be applied to non-Bt corn refuges.

3) For the 2001 growing season, grower agreements (Stewardship Agreements) for Cry1F field corn grown in cotton-growing areas will specify that growers must adhere to the refuge requirements as described in the Grower Guide/Product Use Guide and/or in supplements to the Grower/ Product Use Guide. Specifically, growers in these areas must plant a minimum structured refuge of 50% non-Bt corn. Cotton growing areas include the following States: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Bryan, Caddo, Canadian, Garvin, and Grady), Tennessee (only the counties of Carroll, Chester, Crockett, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Hendersen, Lake, Lauderdale, Lawrence, Lincoln, McNairy, Madison, Obion, Rutherford, Shelby, and Tipton), Texas (except the counties of Carson, Dallam, Hansford, Hartley, Hutchinson, Lipscomb, Moore, Ochiltree, Roberts, and Sherman), Virginia (only the counties of Greensville, Isle of Wight, Northampton, Southampton, Sussex, Suffolk) and Missouri (only the counties of Butler, Dunkin, Mississippi, New Madrid, Pemiscot, Scott, Stoddard).

4) Requirements for refuge deployment will be described in the Grower Guides/Product Use Guides as described in Section D of the Industry IRM Plan submitted on April 19, 1999. Growers must continue to be required to plant only non-Bt corn in the refuge and to plant the refuge within ½ mile of their Cry1F corn acreage. In regions of the corn belt where conventional insecticides have historically been used to control ECB and SWCB, growers wanting the option to treat these pests must plant the refuge within ¼ mile of their Cry1F corn. Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field. When planting the refuge in strips across the field, growers must be instructed to plant multiple non-Bt rows whenever possible.

5) The registrants will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. The registrants will proceed with efforts to develop a discriminating concentration assay. The registrants will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB and corn earworm (CEW) populations to theCry1F protein. This resistance monitoring program will be developed to measure increased tolerance to Bt corn above the various regional baseline ranges.

Populations of ECB and CEW will be collected from representative distribution areas that contain Cry1F corn plant-pesticide and monitored/screened for resistance, with particular focus on those areas of highest distribution. The results of monitoring studies will be communicated to the Agency on an annual basis, by January 31 of the year following the population collections for a given growing season.

In addition, the registrants will instruct its customers (growers and seed distributors) to contact the registrants (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB and/or CEW damage occur.

Upon exclusion of the causes specified in section 7a of this document, the registrants will investigate and identify the cause for this damage by local field sampling of plant tissue from corn hybrids that contain Cry1F corn plant-pesticide and sampling of ECB & CEW populations, followed by appropriate in vitro and in planta assays. Upon the registrant's confirmation by immunoassay that the plants contain Cry1F protein, bioassays will be conducted to determine whether the collected ECB population exhibits a resistant phenotype.

Until such time that a discriminating concentration assay is established and validated by the registrant, the registrant will utilize the following to define a confirmed instance of ECB and/or CEW resistance:

Progeny from the sampled ECB or CEW population will exhibit both of the following characteristics in bioassays initiated with neonates

1. An LC50 in a standard Cry1F diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC50 for susceptible ECB or CEW populations, as established by the ongoing baseline monitoring program. The source of Cry1F crystal protein standard for this bioassay will be *Bacillus thuringiensis* subspecies *aizawai*.

2. > 30% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1F-positive leaf tissue under controlled laboratory conditions.

Based upon continued experience and research, this working definition of confirmed resistance may warrant further refinement. In the event that the registrant finds it appropriate to alter the criteria specified in the working definition, the registrant must obtain Agency approval in establishing a more suitable definition.

The insect monitoring programs must include Southwestern corn borer (SWCB) and corn earworm (CEW), in addition to European corn borer (ECB). The program must focus monitoring in areas that typically have a high density of Bt corn or have historically been prone to high levels of corn borer pressure and where the refuge areas may more likely be treated with insecticides.

6) The current definition of confirmed insect resistance must be used as described in Section E of the Industry IRM Plan. Agency approval will be sought prior to implementation of any modified definition of confirmed insect resistance.

7) a) When field resistance has been demonstrated to have occurred, you must stop sale and distribution of Cry1F corn in the counties where the field resistance has been shown until an effective local mitigation plan approved by EPA has been implemented. The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the 2001 growing season. EPA interprets "suspected resistance" to mean, in the case of reported product failure, that the corn in question has been confirmed to be Cry1F corn, that the seed used had the proper percentage of corn expressing Cry1F protein, that the relevant plant tissues are expressing the expected level of Cry1F protein, that it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been ruled out. The Agency does not interpret "suspected resistance" to mean grower reports of possible control failures, nor should extensive field studies and testing to fully scientifically confirm insect resistance be completed before responsive measures are undertaken.

7) b) The registrant will maintain a (confidential) database to track sales (units and location) of its Cry1F corn on a county-by-county basis. The registrant will provide annually, on a CBI basis, sales data for each state indicating the number of units of corn hybrids that contain the registrant's Cry1F corn plant-pesticide that were sold. As part of the overall sales report, the registrant will provide a listing of an estimate of the acreage planted within such states and counties with sales limitations. This information will be provided by January 31 of the year following each growing season.

8) The registrants will provide grower education. The registrants will agree to include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. The registrants will implement a grower education program (in part, as requested by the registrants, through the Grower Agreement setting forth any resistance management requirements) directed at increasing grower awareness of resistance management, in order to promote responsible product use. Insect Resistance Management educational materials for the 2001 growing season must be provided to the Agency as they become available for distribution. IRM educational materials must be developed and distributed at the same time that growers receive seed. Survey results and other available information must be used to identify geographic areas of non-compliance with insect resistance management plans. As described in the Industry IRM Plan submitted to EPA on April 19, 1999, an intensified grower education program will be conducted in these geographic areas prior to the following growing season. If individual non-compliant growers are identified, they must be prohibited from future purchases of Cry1F corn seed.

IV. Regulatory Position

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient if: 1) insufficient time has elapsed since the imposition of the data requirement for those data to be developed and all other required data have been submitted, 2) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and 3) the registration and use of the pesticide during the conditional registration is in the public interest. BPPD believes that all these criteria have been fulfilled.

The first criterion under FIFRA section 3(c)(7)(C) mentioned above has been met since insufficient time has elapsed since the imposition of the data requirements for:

1) A longer soil degradation study in actual field soil.
2) Confirmatory Monarch butterfly data.
3) Continuation of beneficial insect field monitoring.
4) Insect resistance management data.
5) Analytical methods and method validation for the Cry1F protein in corn have been received and are acceptable, but additional confirmatory methods and standard post-registration EPA laboratory method validation are required.

Although, Cry1F protein plant root expression and exudation data and a 6 week avian feeding study with 60 -70% Cry1F corn in the diet were identified as deficiencies for a non-expiring full commercial use registration, they are not considered data gaps for a registration expiring on September 30, 2001. At this time in the reassessment process, these data have not been required of other Bt corn plant-incorporated protectant registrants.

The applicants have submitted or cited data to satisfy the second criterion for conditional registration under FIFRA 3(c)(7)(C) as mentioned above. Mycogen Seeds and Pioneer HiBred submitted and/or cited satisfactory data pertaining to the proposed use. The human health effects data and non-target organism effects data are considered sufficient for the period of the conditional registration. These data demonstrate that no forseeable human health hazards or ecological effects are likely to arise from the use of the product and that the risk of resistance developing to *Bacillus thuringiensis* during the conditional registration is not expected to be significant. The data also demonstrate that there is virtually no possibility of any risk associated with weediness or outcrossing to wild relatives.

Registration of *Bacillus thuringiensis* subspecies Cry1F protein and the genetic material necessary for its production (plasmid insert PHI 8999) in corn is in the public interest because the new pesticide is comparatively less risky to health or the environment than currently registered pesticides and the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques.

The registered alternatives commonly used to treat the target pest complex protected by Cry1F are restricted use for the most part. They have precautionary label statements such as extremely toxic to fish and aquatic organisms, wildlife and require protective clothing for workers. The specific organophosphate and pyrethroid pesticides likely to be replaced are ranked in the top 15 of all pesticides with respect to reported incidents of mortality to non-target wildlife. Many of these

products also control corn root worm, which is the most significant pest of corn and is frequently treated along with the target pest complex of Cry1F. Compared to other Bt corn products, growers are likely to choose Cry1F protected corn due to better product performance and broader spectrum of control. Cry1F protected corn is also expected to be economical on some unprotected fields and provide insurance against the risk of crop loss and the need to replant. But without root worm protection, the use of Cry1F to reduce conventional pesticide use is limited.

Economic benefits to growers are the incremental improvement to grower profits compared to current practice.

In view of these minimal risks and the benefits BPPD believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient are satisfactory, it is not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data are necessary to evaluate the risk posed by the continued use of this product. Consequently, data requirements specified earlier in Section III were required.

BPPD also believes, as explained in section II.E., that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use of Cry1F corn under this registration is in the public interest.

The related final rule for these registrations involves plant-incorporated protectant Cry1F protein in corn and is found in 40 CFR 180.1217.

In order to link these Cry1F Bt corn registrations to the current Bt crops reassessment process that the Agency is undergoing to ensure that any new necessary modifications to the registration and data requirements that are determined for Bt crops during the reassessment are imposed for these products, an expiration date of September 30, 2001 for the Cry1F products was imposed to match the expiration date of the currently registered Bt corn products being evaluated in the reassessment.

V. Actions Required by Registrants

IRM terms and conditions must be complied with, conditionally required data must be submitted, and reports of incidences of adverse effects to humans or domestic animals and target pest resistance must be submitted under FIFRA, Section 6(a)2.